

## ANALYSIS OF THE REGENERATIVE PROCESSES IN NEMERTEANS

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Because of the sharp demarcation of their tissues, the nemerteans are particularly favorable for a study of the cellular changes which occur during the restoration of the new individual from a fragment of the body. In some species, such as *Lineus socialis*, the regenerative potency is so great that almost any small piece of the body, provided it contains a portion of one of the lateral nerve cords, is able to develop into a minute worm of normal proportions. The persistence of this regenerative ability is demonstrated by repeatedly cutting off portions of partially regenerated pieces until extremely minute individuals less than a hundred thousandth the size of the original are finally obtained (Coe, 1929). The cellular activities involved in such regeneration have already been described (Coe, 1934), and we may now inquire as to the organizing agencies which are responsible for them.

In the regeneration of a fragment of the body of *Lineus socialis* it is evident that the stimulus of the changed internal environment causes a vigorous contraction of the body and a coördinated migration of epidermal and connective tissue cells toward both the cut surfaces. The wounds are thereby closed and healed (Fig. 1).

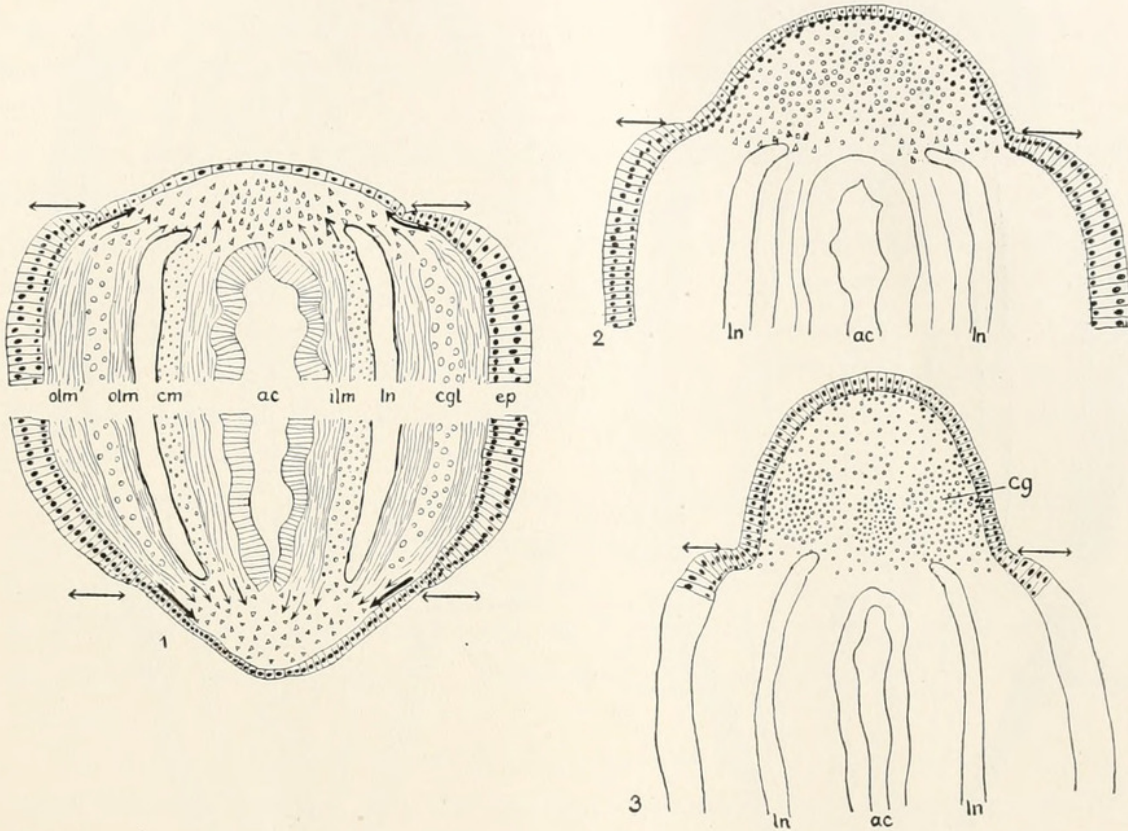
The cuts also activate the dormant cells which are situated in the parenchyma between the organ systems in all parts of the body. By virtue of their activation these cells assume the properties of regenerative cells. They migrate both anteriorly and posteriorly, finally collecting in large numbers beneath the new epidermis which has already covered the cut ends of the fragment.

Those that migrate posteriorly are soon incorporated into the organ systems of the original body. These organs were injured by the cut and their tissues are in process of restoration, both by the multiplication of the differentiated cells and the incorporation of new ones. The fate of each of the migratory cells is presumably determined by the dif-



ferentiated cells which it reaches. The functional tissues thus organize the new posterior extremity.

Posterior regeneration is therefore closely similar to normal growth, although phagocytosis is more extensive in removing unassimilable elements and more essential in the nutrition of the regenerating tissues than is the case in normal growth.



FIGS. 1-3, *Lineus socialis*.

Fig. 1. Diagram of regenerating fragment of body four days after operation, showing migration of epidermal and regenerative cells toward both cut surfaces as indicated by direction of arrows; *ac*, alimentary canal; *cgl*, cutis glands; *cm*, circular musculature; *ep*, epidermis; *ilm*, *olm*, *olm'*, inner and outer longitudinal musculatures, respectively; *ln*, lateral nerve cord. Double arrows indicate positions of cuts.

Fig. 2. Blastema preceding visible localization of organs; sixth day of regeneration; letters as in Fig. 1.

Fig. 3. Blastema showing localization of prospective cerebral ganglia (*cg*) and (in median line) proboscis and sheath; eighth day of regeneration; letters as in Fig. 1.

The regenerative cells which migrate anteriorly, on the contrary, form a true blastema consisting of an apparently undifferentiated mass of mesenchyme cells (Fig. 2). These cells form the basis of an essentially new individual. The blastema resembles the early embryo in that it seems to be a self-determining system, the new organs being



differentiated without regard to the origin of their cells among the parent tissues. It is at present impossible to state which, if any, of the constituent cells of the blastema are derived from the new epidermis which

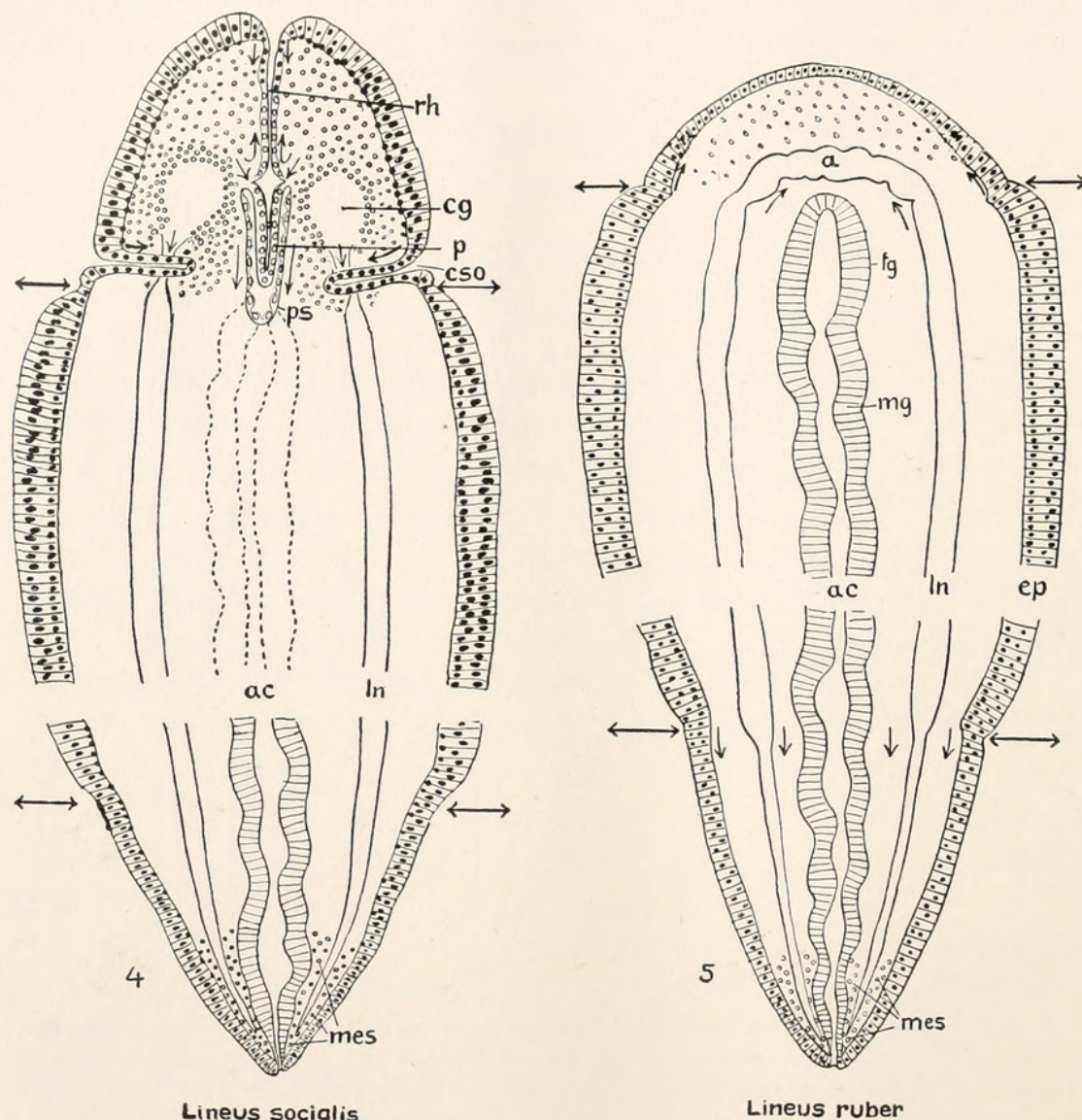


FIG. 4. *Lineus socialis*. Blastema with differentiated primordia of cerebral ganglia (*cg*) with new lateral nerves growing posteriorly to join nerve cords of original fragment; *cs*, invagination of new epidermis to form cerebral sense organs; *rh*, rhynchodeum; *p*, new proboscis growing posteriorly in the new sheath (*ps*). The posterior end shows the elongation of the original organ systems, with migration and incorporation of mesenchyme cells (*mes*). Fifteenth day of regeneration.

FIG. 5. *Lineus ruber*. Portion of body 30 days after operation, showing posterior regeneration only; wound healed at anterior end and nerve cords united, but no blastema is formed; letters as in Fig. 1; double arrows indicate positions of cuts.

covers the mesenchyme. All of the cells appear to be multipotent and capable of differentiation into any of future organs.

Localizations soon become manifest in the blastema and the regen-



erative cells congregate in three principal areas (Fig. 3). Two of these are situated laterally and represent the primordia of the cerebral ganglia, while the median group furnishes the cells for the new proboscis and proboscis sheath.

The cells which are later differentiated into the ganglia are so closely associated with the basal cells of the new epidermis along the lateral margins of the blastema that it seems quite possible that some of them may be of epidermal origin. If so, they may be predetermined or partially determined in the direction of an epidermal derivative such as the nervous system.

Soon after their differentiation the primordia appear to act as induction centers for the other cephalic organs. From the base of the proboscis primordium a group of cells becomes differentiated into a slender tube of epithelium which represents the future rhynchodeum. Simultaneously a group of epidermal cells at the anterior margin of the blastema forms a slender invagination which joins the rhynchodeum. The proboscis is thus brought into communication with the exterior (Fig. 4).

The cerebral ganglia are likewise associated with, and presumably induce, a pair of epidermal invaginations on the lateral margins of the blastema. These represent the future canals of the cerebral sense organs (Fig. 4). The new mouth is formed by another epidermal invagination; this is evidently induced by the anterior end of the new foregut.

As soon as all the organs of the new head have been restored, that is, when the regenerated part has become individualized, it reorganizes all the tissues of the original fragment into units of smaller size. Nutrition by phagocytosis leads to compensatory growth of the new part, the size which is reached before external food is taken being obviously dependent upon the dimensions of the original fragment.

To accomplish this reorganization, the new organs, which have been differentiated in the blastema quite independently of the organs in the original fragment, grow posteriorly and make connections with such of the original organs as are represented in the fragment. The minute new nerve cords join the much larger cords of the fragment, the new mouth joins the old alimentary canal and the new blood vessels unite with the old. Phagocytic activities remove such parts of the old organs as cannot be incorporated into the new and transfer the available materials as nutrition for the new tissues. An essentially new individual of harmonious proportions results.

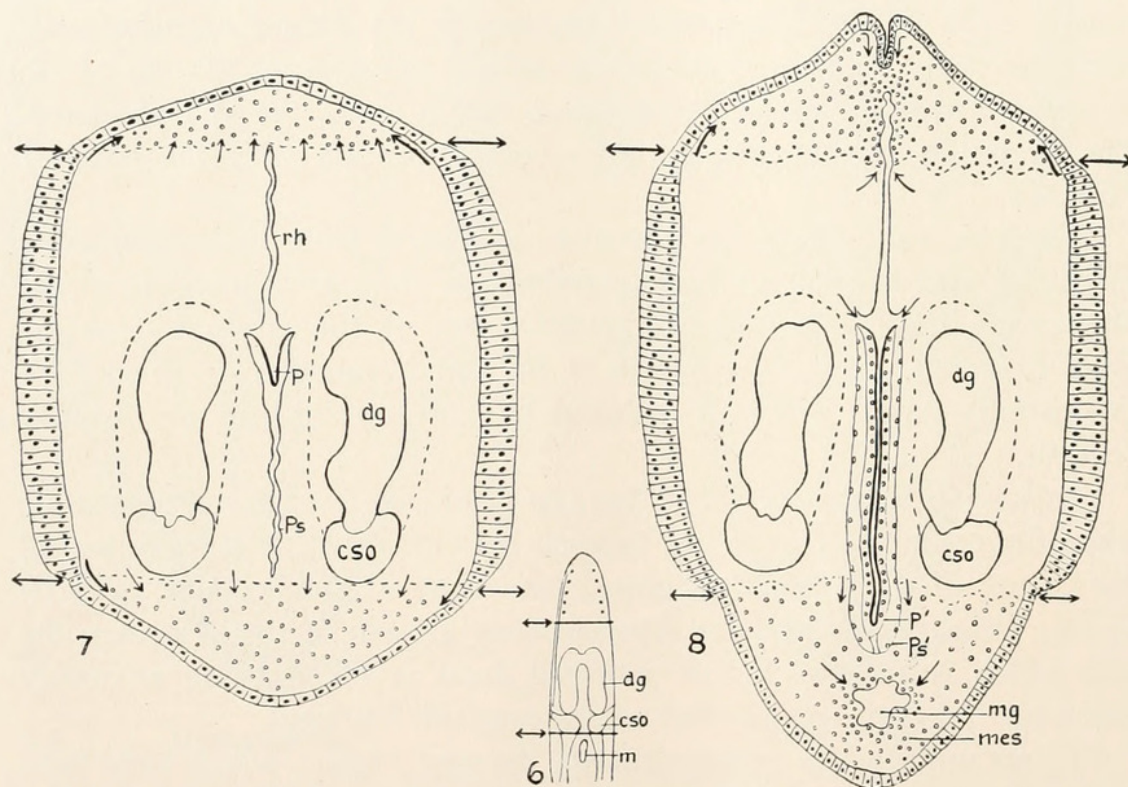
Since the original proboscis is not represented in the fragment the new proboscis, with its sheath, grows posteriorly into the parenchyma



which fills the space formerly occupied by the old proboscis and sheath or in a corresponding position if the fragment was taken posterior to the end of the sheath. During this elongation the walls of the proboscis become differentiated into epithelial, muscular and connective tissue layers, as previously described (Coe, 1934). Nerves grow into the organ from the new brain.

#### REGENERATION OF A NEW BODY FROM A PORTION OF THE HEAD

The conditions are somewhat different when a new body is restored from the head alone or even from a portion of the head, as shown in Figs. 6 to 11. If two transverse cuts be made so as to remove the anterior portion of the head as well as the entire body posterior to the



FIGS. 6-8. *Lineus socialis*. Early stages in regeneration of new body from portion of head, cut as indicated by double arrows in Fig. 6; *cso*, cerebral sense organ; *dg*, dorsal ganglion; *m*, mouth; *mes*, mesenchyme; *mg*, primordium of midgut; *P*, proboscis; *Ps*, proboscis sheath; *rh*, rhynchodeum; arrows show direction of migrating cells.

cerebral sense organs (Fig. 6), the tissues to be replaced anteriorly will be organized by those remaining. If all trace of the digestive system, including the buccal epithelium, has been removed from the posterior end of the head, an entirely new alimentary canal must be replaced. This is accomplished by mesenchyme cells which migrate posteriorly from the remaining cephalic tissues (Figs. 7, 8). These mesenchyme



cells are preceded by wandering phagocytes which congregate at the site of the future midgut to form an irregular mass of cells with more or less tissue fluid between them. The mesenchyme cells then arrange themselves in a single layer around this fluid area to form the primordium of the midgut (Fig. 9). With further additions and by cell division the mesenchyme cells become differentiated into columnar epithelium characteristic of the digestive system (Coe, 1934).

A median outgrowth of the epithelium at the anterior end of the midgut forms the primordium of the foregut. This is followed by an invagination of the new epithelium on the ventral side of the body to form the new mouth (Fig. 10), exactly as in the case of the regenerating body fragments previously described. The foregut, of mesenchymal origin, then forms a communication with the buccal cavity, of epidermal origin, and the constituent cells are mingled.

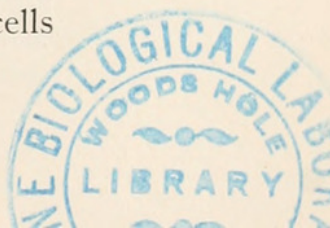
These processes may be interpreted as implying that the epidermal invagination is induced by some stimulus originating in the newly formed foregut, since the epidermal cells appear to be activated only after the foregut has reached a definite stage of differentiation. The posterior end of the midgut is capable of indefinite extension, both by cell proliferation and by the incorporation of mesenchymal cells which are always present at the posterior end of the elongating body (Figs. 9-11).

Reorganization, or regulation, of the old cephalic tissues by phagocytosis supplies sufficient material for the minute new individual which results (Fig. 11).

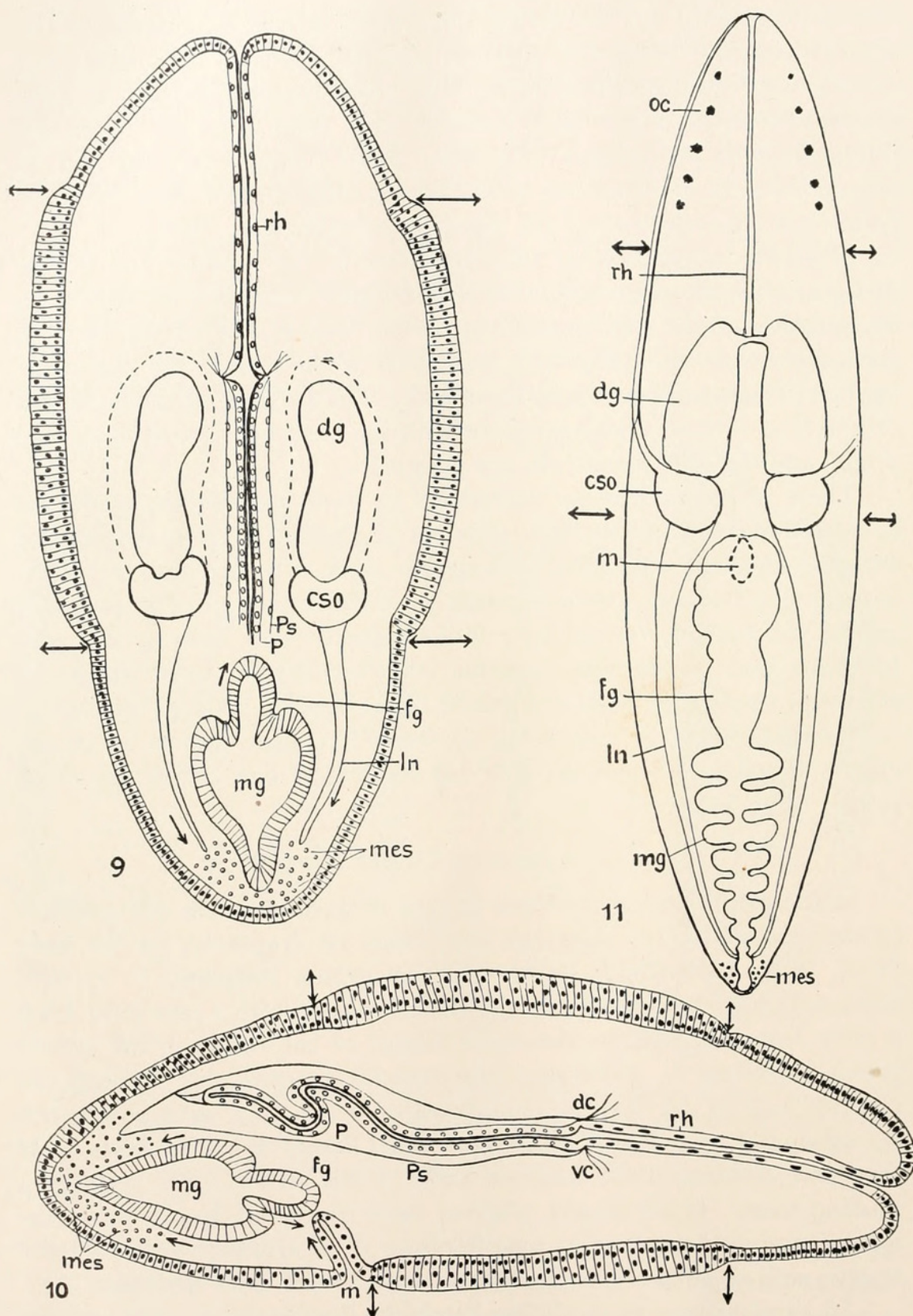
#### REGENERATION IN OTHER SPECIES

In the genus *Lineus* are three groups of species which differ widely in their capacities for restoring new heads on fragments of the body (Coe, 1929, 1930, 1932, 1934). In one group, including *L. socialis*, discussed on the preceding pages, the ability to restore a new head from a body fragment extends the entire length of the body; in the second group, including *L. pictifrons*, this capacity extends only through the anterior half of the foregut region, while in the third group, of which *L. ruber* ("broad form," Nusbaum and Oxner) is an example, only that part containing the anterior ends of the nerve cords restores a missing head. If we inquire wherein these groups of species differ the answer may be found either in differences of distribution of the regenerative cells or in the agencies which may control their activities.

All three groups show similar capacities for wound healing and for posterior regeneration, and the parenchyma in all these species contains cells of similar appearance. In the first group, however, the cells which migrate anteriorly are immediately organized into the regenerative cells of the blastema and hence of an essentially new organism.







FIGS. 9-11. Later stages in regeneration of new body from portion of head (continuation of Figs. 6-8); *fg*, foregut; *ln*, lateral nerve cord; *dc*, *vc*, dorsal and ventral brain commissures; *oc*, ocelli; other letters as in Figs. 6-8; double arrows indicate positions of original cuts. Compare relative volumes of old and new tissues.



In the third group, on the other hand, such cells as migrate anteriorly retain the characteristics of parenchyma cells and as such supply merely the connective tissues and phagocytic cells for the completion of wound healing but without restoring the missing parts. The growth of these connective tissues may be very extensive and the headless fragment may live for several months. During this period the organ systems may be considerably altered anteriorly, often leading to the fusion of the cut ends of the nerve cords in the median line (Fig. 5). But the old organism remains incomplete and eventually dies, presumably because it lacks either the cells necessary for the restoration of a new head or the suitable stimulus for their activation, or both. The possibility of the presence of an inhibiting influence must also be kept in mind. Since the head alone may restore a new body, while the body itself cannot replace the missing head, and since regeneration does not occur unless portions of the nerve cords are present, we may conclude that the organizing, or morphogenetic, agent is in these species limited to the head and more particularly to the anterior ends of the nerve cords.

The region of complete regeneration in any species would thus be coextensive with bipolarity in the movements of the regenerative cells, extending the entire length of the body in individuals of the first group but only to the posterior ends of the cerebral sense organs in the third. An intermediate condition exists in the second group, where this organizing potency extends from the brain to the middle of the foregut region.

In the first group all parts of the body which contain any portion of the nerve cords are equally capable of regeneration and a hundred simultaneously regenerating fragments may be obtained from a single individual. In the second group only two new individuals have been obtained from one, while in the third group no increase in number has been found possible since only that fragment survives which contains the anterior ends of the nerve cords.

Corresponding differences in the regenerative capacities of different parts of the body in oligochætes are attributed by Stone (1932, 1933) to a restricted distribution of the regenerative cells, while Curtis and Schulze (1934) find that the regenerative ability in species of planarians is correlated with the relative number of regenerative cells preformed in the parenchyma.

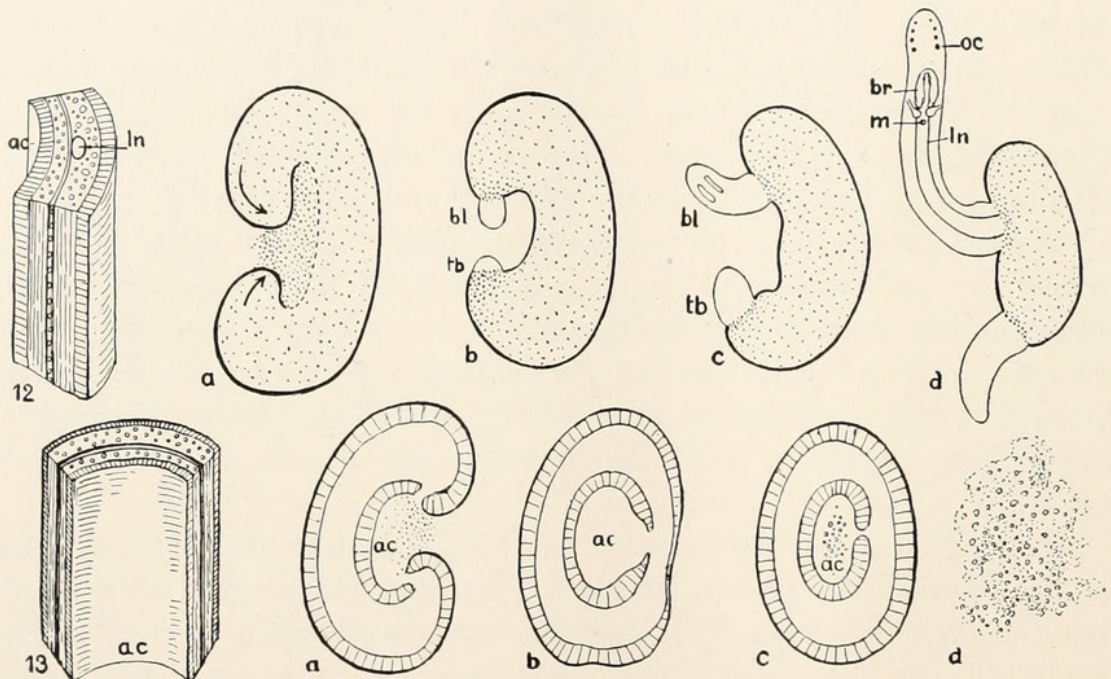
#### INFLUENCE OF THE NERVOUS SYSTEM

It must be kept in mind that the processes described appear to be in some way dependent upon the presence of some part of the nervous system. Repeated observation of body fragments of *Lineus socialis*



indicates that at least a small portion of one of the lateral nerve cords must be present in order that complete regeneration and regulation may take place. Wound healing occurs normally in a fragment lacking any portion of the nerve cords and the remaining tissues may be reorganized extensively, but an anterior blastema is not formed. Such reorganized pieces may remain alive for several weeks but individualization is not accomplished and disintegration has followed in every case observed (Fig. 13).

Whether this lack of regenerative capacity is due to the removal of essential regenerative cells or whether to the absence of some stimulus required by the cells remaining is at present uncertain, but from a con-



FIGS. 12, 13. *Linus socialis*. Fig. 12, regeneration of sector of fragment containing portion of nerve cord (ln); bl, blastema at anterior end of nerve cord; tb, tail bud; oc, ocelli. Fig. 13, reorganization, without regeneration, of sector of fragment lacking any portion of nerve cord; disintegration after a survival of thirty days; ac, portion of alimentary canal.

sideration of all aspects of the regenerative processes in this and other species the evidence seems to favor the latter alternative.

The extent to which this stimulating agent is present along the length of the nerve cords in various species may, conceivably, be responsible for the observed differences in their regenerative capacities. For we must recall that in other, apparently closely related, species a new head is not formed even when the entire nervous system posterior to the cerebral sense organs is intact. Such differences in the regenerative capacities of morphologically similar species throws additional light on this problem of determination.



In fragments capable of regeneration, the blastema invariably makes its appearance at the anterior cut ends of the nerve cords. In fragments consisting of an entire transverse section of the body, the ends of the two nerve cords are brought closer together by the contractions of the musculatures near the cut surface, while in sectors of the body the single nerve cord ends in the recurved anterior end of the fragment (Fig. 12). In both cases the blastema arises in the new plane of symmetry (Figs. 1-3; 12) at the cut ends of the nerve cords because the regenerative cells have migrated to these positions.

In the planarians likewise the blastema usually arises near the anterior cut ends of the nerve cords, although in certain species even fragments cut lateral to the nerve cord show complete regeneration. In such cases, as Beyer and Child (1930) have shown, the head bud usually forms along the median border of the fragment adjacent to one of the lateral branches of the nerve cord or a group of such branches. They regard the central nervous system as an activating factor concerned in localization, and not as a determining factor nor as necessarily essential for the localization of anterior ends in the planarians studied.

There is a similar close connection between the cut ends of the nerve cords and the position of the regenerative buds in both polychæte and oligochæte annelids. As found by Goldfarb many years ago, removing the nerve cord from near the cut surface causes a considerable delay in the regeneration of earthworms (Siegmund, 1928; Kropp, 1933). If the ends of the cords are turned back and held in this position, normal regeneration does not take place (Bailey, 1930; Holmes, 1931). Evidently the migration of the regenerative cells to the cut surface is thereby prevented, although wound healing occurs as usual.

The inhibiting effects of X-rays on regenerative ability in various groups of animals appear to be due to the destruction of the regenerative cells or to changes which render them incapable of activation. Proliferation of the specialized tissues may also be checked, but wound healing is not prevented unless the dosage is very strong (Zhinkin, 1932; Stone, 1932, 1933).

The bilateral symmetry of the blastema in both planarians and nemerteans is not strictly dependent upon the symmetry of the original fragment, for a fragment taken from either the left or right side of the body produces a blastema of normal symmetry (Fig. 12). The migrating regenerative cells are evidently disposed with reference to the new plane of symmetry imposed by the group as a whole. An asymmetrical blastema has been found only when the original blastema has been split lengthwise. If the parts are then kept separated or one of them removed the original symmetry is slowly restored. Splitting the anterior



end of the fragment before regeneration has made much progress causes the formation of a normal blastema on each part (Coe, 1930).

In the case of wound healing as well as in posterior regeneration the making of the cut causes a disturbance of the normal interrelations of the remaining cells, together with changes in their internal environment. The cells are thereby activated and respond adaptively to the new conditions, finally becoming organized into the functional tissues. The situation at the anterior cut surface is at first similar but complications soon arise because the fragment is without individualization. In addition to the necessity of repairing the organ systems represented in the fragment, a head with entirely new organs must be provided. Head formation resembles embryonic development in that the new organs become differentiated from groups of multipotent cells. In regeneration these cells are derived from dormant parenchyma cells which migrate forward to form the blastema as previously described.

In the activation of these cells it may be supposed that the cut nerve cords liberate some influence, not improbably a growth-stimulating substance, which acts specifically upon the dormant cells of the neighboring parenchyma, transforming them into active regenerative cells and directing their movements anteriorly.

Given this initial stimulus the multipotent cells arrange themselves or are arranged into an aggregate endowed with the power of self-determination. The differentiated primordia of the organ systems act as induction centers for the associated parts of the new systems, whereby the descendents of the original regenerative cells encounter one organizing factor after another in the series of regenerative processes discussed in the preceding portion of this paper. Each step in the series induces the one that is to follow as the organization of the essentially new individual proceeds.

#### SUMMARY

1. An attempt to analyze the organizing potencies in the regeneration of fragments of the body in several species of nemerteans leads to the conclusion that the cut nerve cords liberate an agent which activates the dormant cells of the parenchyma and transforms them into regenerative cells. Bipolar migration of these cells leads to complete regeneration.

2. The different regenerative capacities in closely related species may be dependent upon differences in the extent of distribution either of this activating agent or of the regenerative cells. In one group the entire length of the body is included and all parts are equally capable of regeneration; in another group the regenerative potency reaches only to the middle of the foregut region, while in a third group of species it is



limited to the anterior ends of the nerve cords and head-formation is limited to a single transverse plane.

3. The blastema is considered to be a self-determining system comparable to that of the early embryo. The constituent cells are evidently multipotent and capable of differentiation into any of the new organs. Once activated they and their descendants complete the regenerative processes.

4. The primary organization center is evidently associated with that part of the nerve cords which is capable of activating the regenerative cells and of controlling their bipolar migration. Secondary organization centers result as soon as the primordia of the organ systems have become differentiated in the self-determining blastema.

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(A more complete list of papers dealing with regeneration in nemerteans may be found in Coe, 1929, 1930, 1932, 1934.)





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