Size of Fragment and Rate of Regeneration in Planarians

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Introduction

The splendid investigations by Wolff & Dubois (1948) and Dubois (1949) on the cellular material concerned in the regeneration of planarians have again raised the question whether the process of regeneration follows the course of a mass-reaction, as maintained long ago by Loeb. Dubois (1949) has shown conclusively that the neoblasts (totipotent regeneration cells) in the regeneration blastema may have wandered to the wound from faraway regions of the body. The question as to whether the number of cells determines the speed of regeneration can therefore be approached by experiments relating size of the regenerating piece to speed of regeneration. On this point, conflicting statements are to be found in the literature. No clear distinction has, however, been made between two separate problems: the time of first appearance of the regenerated organs and the size of the organ-anlagen at this time; or, put in another way, the speed of determination of the neoblasts and the number of neoblasts available for determination. Only the first problem has been investigated here; the latter will be dealt with in a paper in preparation.

Abeloos (1930) found that, in Planaria gonocephala, in animals of about the same age the rate of regeneration of eyes from a given level of the antero-posterior axis is the same regardless of the size of the regenerating fragment. He also found that young and small individuals regenerate eyes faster than bigger and older ones. Abeloos holds that age or other physiological conditions and not merely size of regenerating fragment is important for the rate of regeneration. Buchanan (1933) also reported that small segments regenerate at the same rate as big ones; whereas Scharoff (1934) observed that small segments regenerate faster than bigger ones.

A reinvestigation of the relation of size of regenerating piece to speed of regeneration has been made with four planarian species: Dendrocoelum lacteum, Bdellocephala punctata, Euplanaria lugubris, Polycelis nigra.

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MATERIAL AND METHOD

The animals were captured under stones on the shores of Lake Furesø near Copenhagen. The experiments were carried out in a uniform way. The animals were kept in Petri dishes containing 50 ml. of tap-water at room temperature. Each dish contained 10 animals, the water was changed every day, and there was no feeding. Regeneration was regarded as having taken place when eye spots could be seen at a magnification of 25 times with a standard illumination.

TEXT-FIG. 1. Dendrocoelum lacteum. Five types of operation. In all, the head was removed by an anterior cut. In b–e, varying amounts of the posterior end of the body were removed by a second cut.

TABLE 1

Percentage regeneration of Dendrocoelum lacteum

<table>
<thead>
<tr>
<th>Hours of regeneration</th>
<th>Type of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>96</td>
<td>11</td>
</tr>
<tr>
<td>117</td>
<td>88</td>
</tr>
<tr>
<td>140</td>
<td>100</td>
</tr>
</tbody>
</table>

The types of operation are shown in Text-fig. 1. Thirty animals in each group.
RESULTS

_Dendrocoelum lacteum_

One hundred and fifty animals were operated on, 30 animals in each of the five ways indicated in Text-fig. 1. The results are given in Table 1. Regeneration starts simultaneously and is terminated at the same time after all five types of operation. The rate of regeneration is therefore independent of the size of the regenerating piece.

_Bdellocephala punctata_

Thirty animals were cut as indicated in Text-fig. 2, operation _a_, and 30 as in

![Text-fig. 2. Bdellocephala punctata. Two types of operation.](image)

Text-fig. 2, operation _b_. This experiment was performed to see whether severe wounds calling upon a great number of neoblasts might retard the regeneration of eye spots. The results indicate that this is not the case. After 133 hours both groups show 33 per cent. regeneration. After 154 hours the figures were 70 per cent. after type _a_ operation and 63 per cent. after type _b_. After 179 hours all living animals, 28 in each group, had reached 100 per cent. regeneration.

This experiment therefore shows that the rate of regeneration is not affected by increasing the severity of wounding in this way.

_Euplanaria lugubris_

Eighty animals were cut as indicated in Text-fig. 3, _a_. The anterior cut was made behind the eyes so that the distance from the cut to the eyes was the same as the distance from the eyes to the front end of the animal. Forty of the animals were again cut as indicated in _b_, the segments thus cut off ("_b_-pieces") being of the same length as the separated heads; the _b_-pieces were very short in com-
parison with the decapitated animals left after operation \( a \) (‘\( a \)-pieces’), with correspondingly fewer neoblasts. Furthermore, whereas the \( a \)-pieces only regenerate heads, the \( b \)-pieces also regenerate tails from the caudal wound, laying therefore a heavier tax on their neoblasts. Nevertheless, the rate of eye regeneration was the same in \( a \)-pieces and in \( b \)-pieces. After 116 hours all animals in both groups had regenerated eyes. The size of eyes and of regeneration blastemas is larger, however, in the \( a \)-pieces. Detailed data on size differences will be given in a subsequent paper.

**Text-fig. 3. Euplanaria lugubris.** Two types of operation. In \( a \), the head is removed. In \( b \), the posterior part of the body is also removed by a second cut.

**Polycelis nigra**

This planarian is characterized by a string of eyes just within the border of the body in its anterior third. The head frequency curve has been worked out for the population in Lake Furesø near Copenhagen (Brøndsted, 1942). The time-graded field has not yet been studied in detail, but in analogy with *Bdellocephala punctata, Dendrocoelum lacteum*, and *Euplanaria lugubris* it seems safe to conclude that the head frequency curve coincides with the antero-posterior axis of a time-graded field in *Polycelis*; that this is so has been partly shown (unpublished). If this conclusion holds, the time required to regenerate eyes increases from the fore part of the body to the hind end. It is therefore imperative to make the transverse cut at a standard level, and this can best be done at the level just behind the eye string, which was accordingly used in the present experiments.

If the regeneration from the anterior surface of the transverse cut proceeds normally, the first appearance of eyes occurs on the sides of the base of the more or less conical regeneration blastema (Text-fig. 4a). In several animals, especially short pieces, the wound contracts in the manner indicated in Text-fig. 4b. In these no regeneration takes place, the probable explanation being, as pointed out by one of us (Brøndsted, 1939), that the anteriorly oriented gradients from both sides of the body oppose one another and thus mutually inhibit the formation of blastemata.
Eighty animals were operated upon. A transverse cut was made just behind the eye string, as shown by a in Text-fig. 5. Forty of these animals constitute group a. In the 40 remaining animals, making group b, the posterior end of the body was also removed by a second transverse cut 0.5 mm. farther back, at b in Text-fig. 5. Six days after the operation the eyes began to appear. The number of individuals with eye spots regenerated were counted 145 hours after operation. In group a, 34 individuals had survived, and 23 (68 per cent.) had regenerated eyes. In group b, only 18 specimens survived, but 15 (83 per cent.) had regenerated eyes. It can therefore be concluded that in Polycelis also the size of the regenerating body segment is not important for the rate of regeneration.

**DISCUSSION**

Dubois (1949) has conclusively shown that neoblasts forming the blastema are derived not only from neighbouring regions of the wound but also, if necessary, from distant parts of the body. It is tempting to conclude that the rate of
regeneration is dependent on the number of neoblasts wandering to the wound. But if that were the case one would expect to find a difference in the rate of regeneration in large and small fragments. We did not do so. The assumption is made further improbable by the fact that short transverse fragments (in addition to providing for the head-forming blastema) also provide neoblasts for the regeneration of the hinder end of the body.

The experiments were done on four different species, none of which propagate by fission. Our results are not therefore influenced by factors arising from physiological processes due to asexual formation of new individuals, as for instance is the case in *Planaria dorotocephala*. We consider that the species here used furnish more reliable results than do species with fission.

Only when, as in the experiments of Dubois (1949), the neoblasts in the piece which is to regenerate are killed by X-rays is regeneration retarded. This is because no neoblasts are immediately available for blastema formation; they have to migrate from distant non-irradiated parts of the body.

**SUMMARY**

Neither size of regenerating piece nor extent of wound influences rate of regeneration of eyes from a given level of the body in four species of planarian.

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**REFERENCES**


