Influence of Temperature on Rate of Regeneration in the Time-graded Regeneration Field in Planarians

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INTRODUCTION

There exists in planarians a time-graded regeneration field for head regeneration (Brøndsted, 1946, 1956; A. & H. V. Brøndsted, 1952). The characteristics of this field, expressed by rate of regeneration, are species-specific. The existence of this field ensures harmonious regeneration from cuts everywhere in the body, as a cut will always expose a 'high point' where regeneration of a head starts with greatest speed, thus taking the lead in organization and at the same time inhibiting head-forming tendencies elsewhere in the blastema (Brøndsted, 1956).

The factors underlying these characteristics of the field are unknown; the problems involved are being attacked from several angles in our laboratory. For the sake of this work it is of some interest to know how the different rates of regeneration at various levels in the time-graded fields might be influenced by various temperature levels.

MATERIAL AND METHODS

The experiments were carried out on two species differing greatly in the characteristics of their time-graded regeneration fields.

*Euplanaria polychroa* (Text-fig. 1) belongs to a group of species characterized by ability to regenerate a head from almost every part of the body, but at rates falling off evenly caudad and laterad (A. & H. V. Brøndsted, in preparation). *Bdellocephala punctata* (Text-fig. 2) has a time-graded regeneration field with a high point just behind the eyes; from here the rates fall off rather steeply to a level in the body just in front of the pharynx.

The temperature-gradient chamber of A. Krogh was used. It consists of a box approximately 100 × 30 × 30 cm. made of galvanized sheet, insulated by soft plates made of compressed fibre-board. The box is divided into several compartments by watertight walls likewise made of galvanized sheet. When ice is placed in the first chamber and water in the rest, a temperature gradient is established ranging from about 1-6°C in the chamber close to the ice chamber to about 16°C in the farthermost chamber, when the room temperature is 20–22°C. The lid, likewise insulated, is pierced by holes through which thermometers are

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fitted dipping into the water of the chambers. In our experiments the ice was renewed every day at 8 a.m. and 8 p.m. The temperature stability in the compartments is fairly good, the variation not exceeding \( \pm 1^\circ C \). The animals were placed in 200-ml. glass jars, floating in the water of the chambers. A control of a sort was established by putting animals in an incubator regulated to \( 20\pm0.2^\circ C \) in general use for planarian regeneration in our laboratory.

Head-regeneration was regarded as fulfilled when eye-spots could be discerned in the binocular microscope at a magnification of 16 times with standard illumination.

**RESULTS**

*E. polychroa.* Sixty-five specimens were cut as indicated in Text-fig. 1. Both the *A*-pieces and *B*-pieces were exposed to the following temperatures: \( A_1 \) and \( B_1 \) to \( 20\pm0.2^\circ C \); \( A_3 \) and \( B_3 \) to \( 13\pm1.0^\circ C \); \( A_5 \) and \( B_5 \) to \( 10.9\pm0.9^\circ C \); \( A_4 \) and \( B_4 \) to \( 8.3\pm0.7^\circ C \); and \( A_5 \) and \( B_5 \) to \( 5.1\pm0.5^\circ C \). The mortality was only slight—one or two specimens in a few batches. The duration of the experiment was 2 months. Text-fig. 3 gives the percentage of head-regeneration with time. The specimens were examined every morning.

There are three salient features. (1) As was, of course, expected, lower
temperatures have a retarding effect on the regeneration rate. (2) In each batch the spread of regeneration rates is more pronounced at lower temperatures, and the more so in the ‘weaker’ B-pieces. (3) Lowering of the temperature has a comparatively greater retarding effect on regeneration rate in ‘weaker’ parts of the field. The relationship of these features will be discussed later.

Text-fig. 3. *E. polychroa*. Rate of regeneration of A-pieces (full lines) and B-pieces (broken lines) at various temperatures.

*B. punctata*. The animals were cut as indicated in Text-fig. 2, so that the time-graded field was cut at a high level of the field in the A-pieces and at a rather ‘weak’ level in the B-pieces. A hundred specimens were operated upon and were divided into five batches of 20 each. A₁ and B₁ were exposed to 20±0·2°C.; A₃ and B₃ to 16±1·0°C.; A₄ and B₄ to 11·8±1·0°C.; A₅ and B₅ to 1·6±0·4°C. Text-fig. 4 gives a survey of the results, which show the same three features already noted in *E. polychroa*. There was no regeneration of heads at a temperature of 1·6°C.

Text-fig. 4. *B. punctata*. Rate of regeneration of A-pieces (full lines) and B-pieces (broken lines) at various temperatures.
One point must be stressed before evaluating the results. In cutting the animals at an intended level it is impossible to be exact, because even when slightly anaesthetized, the animals are contracted to a varying degree; the cuts will therefore be placed sometimes a little anterior to the intended level, sometimes a little posterior in the time-graded field; for this reason a slight spread of regeneration rate will occur in each batch. In addition, the different specimens undoubtedly show a slight variation in the rate of regeneration at a certain level. This spread has to be taken into account when interpreting the results. When the cuts are made just behind the eyes in *Bdellocephala* they strike a region where the field slopes only slightly, so that one would expect the spread to be rather small; whereas cuts in a region where the field is sloping more steeply must give a greater spread in a batch. The results of the experiments bear this out clearly, as seen in Text-figs. 3, 4, 5, and 6.

The first question to be considered is this: Are our findings consistent with the well-known gradient hypothesis of Child? Or do they confirm the findings of E. Løvtrup (1953) and Pedersen (1956), that no respiratory gradient exists, in either the normal or the regenerating animal? Child’s hypothesis has been discussed in some detail by Brøndsted (1955).
It is, of course, easy to see that lowering the temperature will slow the regeneration processes, as these obey general physiological laws. But if a respiratory gradient existed in the animals, conforming to the time-graded regeneration field, then it seems to us that the regeneration rate would be depressed in the same way as the rate of a respiratory gradient, that is, the curves seen in Text-figs. 5 and 6 would run parallel. We therefore have to look for other explanations of the fact that the regeneration of the B-pieces is depressed more than that of the A-pieces. In this connexion it must be stressed that tail-regeneration and in fact all other regeneration than that of a head is very good everywhere in the body in both species. It is therefore not the regenerative power as such which follows the time-graded regeneration field of head-formation. It seems to us that the most plausible interpretation of our results is that special mechanisms are distributed throughout the planarian body in such a way as to be necessary and responsible for head-formation. This means that such mechanisms decline in amount posteriorly and therefore demand more time for expressing themselves in head-formation. Therefore the lower the temperature the greater the discrepancy in time which must ensue between regeneration from anterior and posterior parts of the body. Text-figs. 5 and 6 bear this out. These curves are drawn between points indicating 50 per cent. regeneration in each batch.

Table 1 gives the temperature coefficients for regeneration rates for intervals...
of $10^\circ C$. throughout the entire range of temperature in which the experiments have been carried out. It is interesting to compare these coefficients for the two contrasting species. The exact validity of the coefficients must admittedly not be stressed too much, since the experimental conditions do not warrant this, but none the less we are confident that the experimental uncertainties are not

**Table 1**

*Mean regeneration times of heads at two levels in the time-graded field at various temperatures*

The coefficients are given for intervals of $10^\circ C$.

<table>
<thead>
<tr>
<th>Temperature ($^\circ C$)</th>
<th>A-pieces</th>
<th>B-pieces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean time of regeneration (days)</td>
<td>Coefficient</td>
</tr>
<tr>
<td>20</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>12.2</td>
<td>3.4</td>
</tr>
<tr>
<td>19</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5.4</td>
<td>4.1</td>
</tr>
<tr>
<td>7</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>5.8</td>
<td>4.6</td>
</tr>
<tr>
<td>6</td>
<td>27.0</td>
<td></td>
</tr>
</tbody>
</table>

| Mean 3.6                  | Mean 3.5 |

<table>
<thead>
<tr>
<th>B. punctata:</th>
<th>A-pieces</th>
<th>B-pieces</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>4.6</td>
<td>6.2</td>
</tr>
<tr>
<td>10</td>
<td>13.2</td>
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<tr>
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<td>12.2</td>
</tr>
<tr>
<td>7</td>
<td>17.9</td>
<td>52.8</td>
</tr>
</tbody>
</table>

| Mean 2.7     | Mean 4.0 |

greater than the minimum required to bear out the general trends as seen in the figures. In the hands of experienced workers, batches of 10–15 specimens have been proved to be sufficient in experiments for working out the extension of the time-graded field in several species. In our experiments the two species behave differently in two respects. In *E. polychroa* (Table 1) there is a marked difference between the A- and B-pieces in so far as the A-pieces are influenced more strongly by lowering the temperature; such a difference is not found in *Bdellocephala*, where the coefficients are nearly the same at all temperature levels in
the A-pieces. In *E. polychroa* the mean of the coefficients at all temperature intervals is the same in A- and B-pieces, whereas in *Bdellocephala* there is a marked difference between the A- and B-pieces in this respect, the B-pieces being much more retarded than the A-pieces. It is, of course, impossible yet to interpret the results adequately; we are, however, inclined to believe that in *E. polychroa* the mechanisms responsible for head-formation are not so highly concentrated in the anterior region of the body as in *Bdellocephala*. Lender in various papers (1950–6) and Török (1958) have shown that the brain and other nerve-cells act as inducers for eye-formation. It seems rather probable therefore that the number of nerve-cells might be responsible for the rate of head-regeneration as detected by eye-formation. A clear-cut task would therefore be to investigate whether the contrasting power of head-regeneration in the two species used for our experiments depends on differences in the number of nerve-cells. Such an investigation is in progress in our laboratory.

However, the temperature experiments have shown another important feature. It was found that at lower temperatures the blastemata were formed a long time before the first eye-spots could be detected. This means that the wandering of the neoblasts to the wound was not seriously impeded at low temperatures. This is in accordance with the fact that both species at low temperature are very much alive in their natural surroundings in Lake Fureso near Copenhagen, where we have collected the animals. We have found newly laid cocoons at temperatures from 2 to 6° C. What is impeded by lowering the temperature is therefore the biochemical processes necessary for cellular differentiation.

At low temperatures, the strong retardation or complete stoppage of processes of differentiation necessary for fulfilment of regeneration makes the hypothesis of the adaptive value of regeneration doubtful; *Bdellocephala*, for example, has its egg-forming and egg-laying period from November to March, and the young are generally only hatched in April and May. So the *Bdellocephalas* have spent the better part of their lives at temperatures which impede regeneration.

**SUMMARY**

1. The aim of the experiments was to see how different levels in the time-graded head-regeneration field of two species, differing strongly as to the characteristics of the field, might behave at different temperatures.
2. As was expected, regeneration was retarded for all levels by lowering the temperature.
3. Regeneration of heads was retarded relatively more strongly at posterior levels of the field.
4. Some characteristic differences in regeneration rates of the two species were found, leading to certain suggestions as to the possible nature of the field.
5. By lowering the temperature regeneration may be separated into two main components: formation of the blastema and differentiation of the cells.
6. Formation of the blastema is not seriously impeded at temperatures below 5–6°C, whereas the differentiation processes are nearly brought to a stop at these low temperatures.

7. Doubt is therefore cast on the hypothesis of an adaptive value of regeneration in view of the fact that several planarian species actually live and reproduce at temperatures well below those necessary for regeneration.

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REFERENCES


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