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EXPERIMENTS ON THE
TIME-GRADED REGENERATION FIELD
IN PLANARIANS

WITH A DISCUSSION OF ITS
MORPHOGENETIC SIGNIFICANCE

BY

H. V. BRØNDSTED



København 1956
i kommission hos Ejnar Munksgaard

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Synopsis.

1. Transplantation experiments have shown that the time-graded head regeneration is a physiological property locally fixed to different parts of the planarian body. (I).
2. The bearing hereof on the problem of bilateral symmetry has been elucidated by means of median splitting and retransplantation at various levels. (II).
3. The significance of the existence of a time-graded field has been emphasized by the performance of twin parabioses. (III).
4. The spreading of inhibitory forces emanating from the highpoint has been clarified. (IV).
5. The starting rate of head regeneration is dependent on the place in the time-graded field; later quantitative growth of head structures is dependent on the number of neoblasts present. (V.)
6. The restoration of the time-graded field is conform with the morphological remoulding of the animal during morphallaxis. (VI).
7. A hypothesis is suggested covering common principles in embryogenesis and regeneration. (VII).

Introduction.

The existence of a time-graded regeneration field in planarians has long been established through extensive studies. Its structure and dynamics are, however, far from being explained satisfactorily. With the aim to elucidate some of the problems, certain experiments have been carried out and will be discussed in this paper.

Earlier experiments on *Bdellocephala* and *Dendrocoelum* (Figs. 1 *a*, *b*) have shown that every part of the body anterior to the pharynx has the power, time-graded in a certain way, to regenerate a head, if an anterior surface is exposed by a cut. The head regeneration rate confirms with a field as depicted in

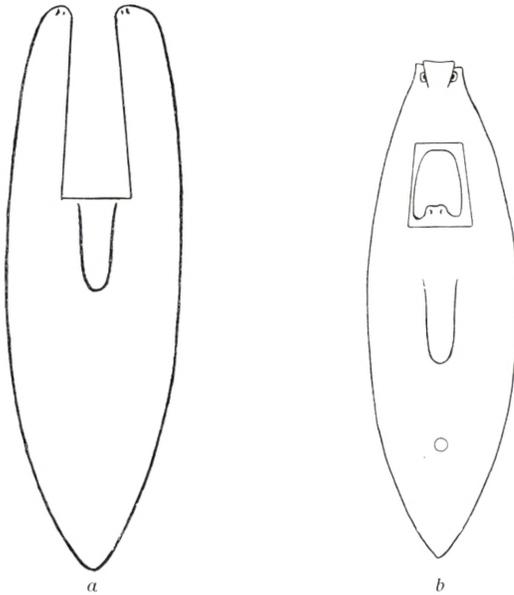


Fig. 1. *a*. *Bdellocephala punctata* regenerating heads on each of two "arms" after decapitation and excising of a median part of the body. *b*. *Bdellocephala punctata* regenerating a head in a "window" cut out from the anterior part of the body. (BRÖNSTED 1942, 1946.)

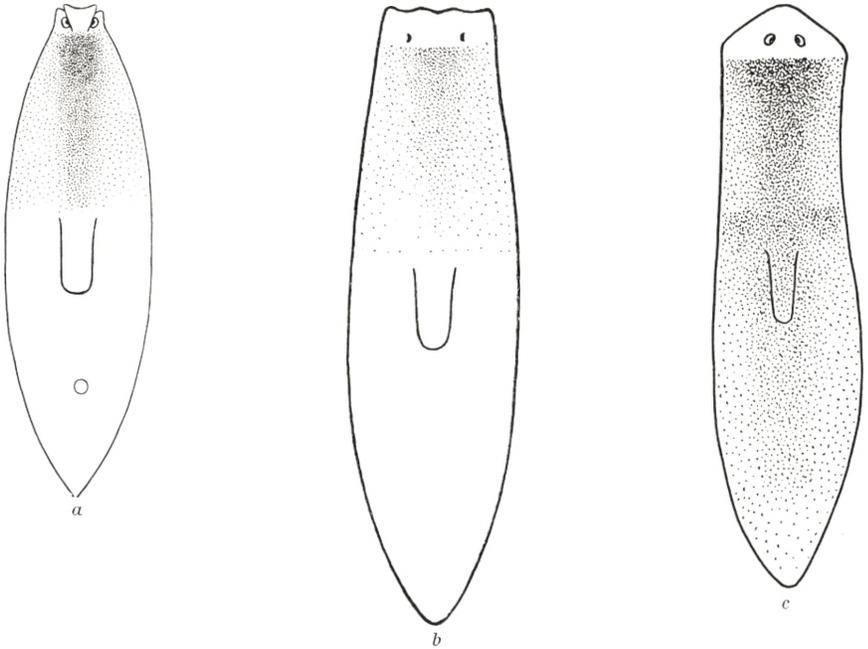


Fig. 2. The time-graded head regeneration fields of a, *Bdellocephala punctata*, b, *Dendrocoelum lacteum*, and c, *Euplanaria lugubris*. (BRØNDSTED, 1942, 1952.)

Figs. 2 a, b (BRØNDSTED 1942, 1946). Experiments on *Euplanaria lugubris* (A. and H. V. BRØNDSTED, 1952) indicate that the time-graded regeneration field extends over the whole body (Fig. 2 c). The field manifests itself when a wound surface is exposed and regeneration starts. I have denoted it a static and potential field.

The time-graded regeneration field secures that only one head is formed on a transverse cut, inspite of the fact that every part of the wound has the ability of regenerating a head. This may be explained by the assumption that an inhibitory influence is exerted by that part of the wound which is regenerating at the most rapid and vigorous rate. In view of its high regeneration rate this part will be called the *high-point*.

Harmonious regeneration seems to rely on two main factors, one of them organizing the totipotent, embryonic neoblasts which form the blastema, the other one inhibiting identical regeneration processes in other neoblasts in the lateral parts of the blastema. Thus, both factors compete in the blastema for determination and differentiation of the neoblasts.

I. Stability of the Time-Graded Field.

This problem was studied by means of a transplantation technique (BRØNDSTED 1939); a preliminary report of the results has been given elsewhere (BRØNDSTED 1954).

Euplanaria lugubris. Several animals were operated upon as shown in Fig. 3. With the aim to find out whether the time-graded field is dependent on humoral or structural factors in the body segments, the median segment, *a*, which in its proper place has a higher rate of regeneration (cf. Fig. 2 *c*), was interchanged with a lateral segment, *b*.

In Figs. 4 *a-c*, it is seen from the unpigmented demarcation lines that the transplanted median segments have retained their bilateral symmetry and have regenerated eyes at the same rate as they would have done prior to transplantation, i. e. 100 hours after the operation distinct eyes had been newly formed. On the other hand, the lateral segments, although implanted in the medial line of the body, have not reached the high regeneration rate characteristic of median segments. The three specimens exhibit a somewhat different behaviour. In *a*, after about ten days, an eye appeared in front of the right side of the body, making it probable that it had regenerated from the right part of the body and not from the transplanted lateral segment with which it has formed a common blastema. Here, the power to regenerate eyes seems to be inhibited (cf. Section IV). In *b*, the transplanted

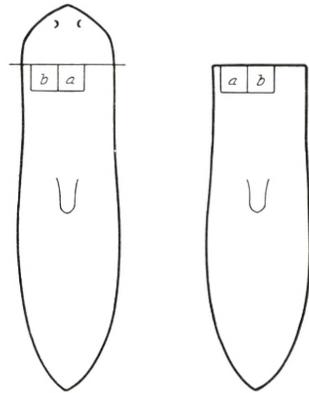


Fig. 3. *Euplanaria lugubris*. The animals were decapitated and the median *a*-piece was exchanged with the *b*-piece.

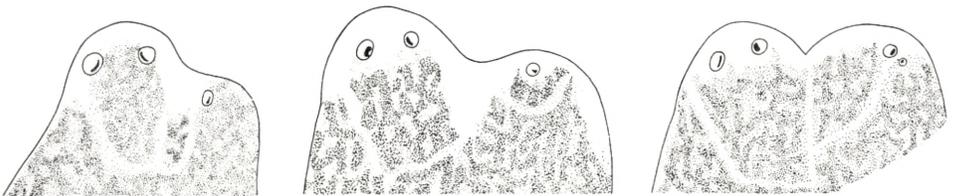


Fig. 4. *Euplanaria lugubris*. *a-c*. Three instances of the exchange experiments the principle of which is seen in Fig. 3. For details, see the text.

lateral segment has formed its own eye after seven days; in *c*, the lateral segment has regenerated an eye after eight days and, at the same time, the right body segment has formed an eye in the course of ten days.

The experiments seem to indicate that the time-graded field is dependent on some structural factor residing in the body parts. They also show that *bilateral symmetry is not attained by the*

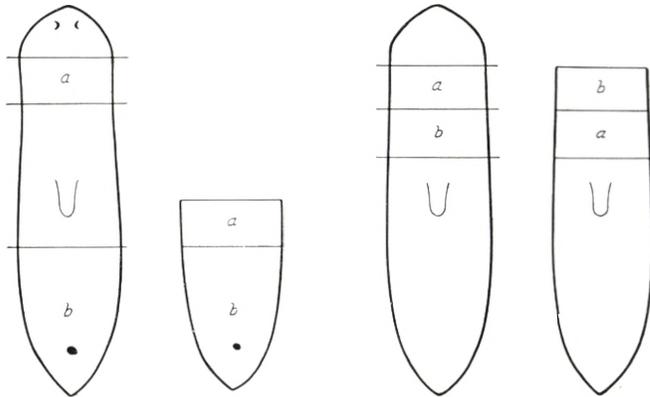


Fig. 5.

Fig. 6.

Fig. 5. *Euplanaria lugubris*. After decapitation, section *a* is transplanted to *b*; it retains its rate of regeneration.

Fig. 6. *Euplanaria lugubris*. After decapitation, section *a* is exchanged with section *b*, which regenerates a head at its inherent rate.

lateral segments when transplanted into the medial line of the body (cf. Section III).

Fig. 5 indicates the way in which *Euplanaria lugubris* was operated upon in order to elucidate whether the field is fixed also after being removed from its original cranial position to a caudal one, where the rate of regeneration is much lower (cf. Fig. 2 *c*). From 20 experimental animals six survived. They regenerated eyes after about 100 hours, which is the time required for eye formation at the original level in the normal animal.

From Fig. 2 *c* it is seen that the rate of regeneration from cuts made in the time-graded field almost in the middle between the eyes and the pharynx is somewhat lower than from cuts made just behind the eyes. 20 animals were operated, as indicated in Fig. 6, in order to find out whether the regeneration rate of more posterior segments of the body can be accelerated by

transferring them to anterior parts of the body. In fact, they regenerate eyes at the same rate as they do in segments left at their normal place. 120 hours after the operation, only four out of seven living animals had regenerated small eye spots, while all seven had regenerated eyes after 140 hours. (The mean rate of regeneration at this level has earlier been determined to 130 hours).

In *Bdellocephala punctata*, only the part of the body anterior

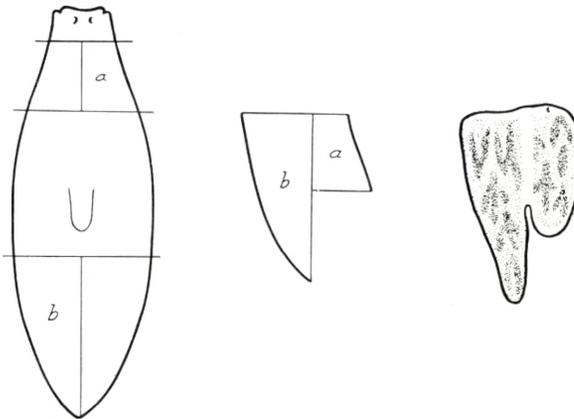


Fig. 7. a. *Bdellocephala punctata*. After decapitation, section *a* was transplanted to section *b*, where it regenerated a right eye; no eyes were regenerated from *b* in the common blastema, the tissue in *b* having no eye-forming competence.

to the pharynx has the ability to regenerate a head (cf. Fig. 2 *a*). In order to get information about whether also in this species the field is inherent in a given segment, irrespective of its situation in the body, two types of experiments were performed. In the first series (Fig. 7 *a*) 20 animals were operated upon as indicated; only one chimera survived, showing that the two pieces had confluent anterior and posterior blastemata. After 90 hours, one eye had been regenerated in the blastema of the piece *a* before the chimera cytolized, thus requiring the normal time for eye regeneration at the respective level in non-transplanted segments.

The second experiment was carried out in order to learn whether polarity in both the segment and the field is retained even if they are exposed to the action of a segment with opposite polarity. 40 animals were operated upon as indicated in Fig. 7 *b*.

On the first day, cuts I—III separated two segments of the body from the head and the tail and from one another; the following day, when the wounds had healed, new cuts bisected the segments, and pieces *a* and *b* were combined with reversed polarity. Two days after the operation, only one chimera survived, pieces *a* and *b* cohered only at about half of the medial line; again two days later the free parts of the chimera were removed. After six

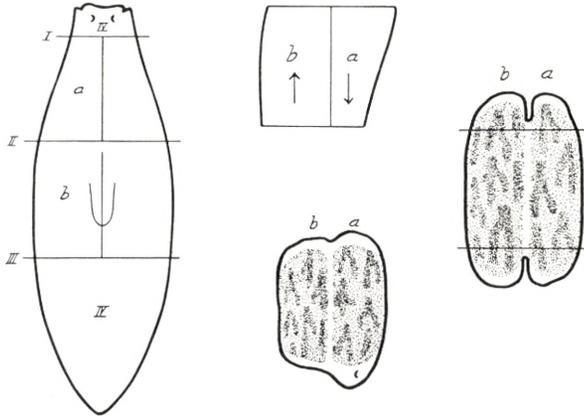


Fig. 7. *Bdellocephala punctata*. After decapitation, section *a* was transplanted to section *b* with reversed polarity; after some time, two clean transverse cuts were made as indicated. Hereafter, *a* regenerated a left eye, whereas *b* did not regenerate eyes in spite of common blastema with *a*.

days, piece *a* had formed a left eye in the left side of its blastema, which however is confluent with the blastema of piece *b*; the latter regenerated a tail; a tail was also developed in the caudal part of piece *a*, from a blastema confluent with the anterior blastema of *b* which does not regenerate eyes.

Thus, the two parts retain their polarity and field, irrespective of the close contact with one another. Actually, they behave as if they were belonging to two different organisms.

II. The Bilaterality of the Field.

In 1946, it was shown (BRØNDSTED 1946) that the regeneration of lateral segments starts at the same time as the regeneration of the organs belonging to the respective lateral part. A left piece starts, e. g., with the formation of a left eye, only later does a

right eye develop (Fig. 8). If a decapitated animal is cut into halves by a median cut, and separated, each half regenerates first its "own" eye; the symmetrical eye appears later and the other half of the body is regenerated subsequently. Thus, in fact, the bilateral animal is made out of two onesided animals acting together and controlling each other's ability to form a new symmetrical half. This example of inhibition may conceal a good deal of the morphogenetic riddle.

In view of the time-graded and bilateral antero-caudal property of the field, interesting results may be obtained by separating the field medially into two longitudinal halves and transplanting them together with the two half-fields transposed along the median axis.

In a series of 60 *Euplanaria lugubris*, the animals were separated by a median longitudinal cut, and the two halves were transplanted together in varying positions. After separation, the pharynges had to be removed, since they were protruding and prevented a proper coherence of the two halves; rather few animals survived.

The procedure involves certain difficulties, one of which may be mentioned here. Due to muscular contraction, the halves tend to curve so that the longitudinal wound becomes concave; therefore, transplantation requires repeated readjustments. Nevertheless, several specimens may contract so strongly as to overlap, with the result that they cohere at the wound surface only slightly or not at all.

A separated half will regenerate a new head after decapitation (Fig. 8). A left half will first regenerate a left eye and after a couple of days a right one which, of course, is smaller than the left one in the beginning. This experiment may serve as a control of the transplanted half, showing the regeneration pattern of an isolated half which has no possibility of cooperating with the symmetrical field.

Example a. One of two animals was separated and the two

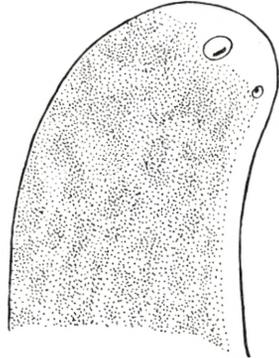


Fig. 8. *Euplanaria lugubris*. After decapitation and median splitting, the left half of the specimen first regenerated a left eye, only later the symmetrical right eye.

halves were joined (Fig. 9 *a*), transposed about $\frac{1}{4}$ of the length of the animal. After three days, the chimera was decapitated by a cut (as indicated in Fig. 9 *a*), which met the right time-graded field at a level of fast head regeneration, whereas the left field was met at a level with a lower regeneration rate. Seven days after decapitation, the right half had regenerated one large right

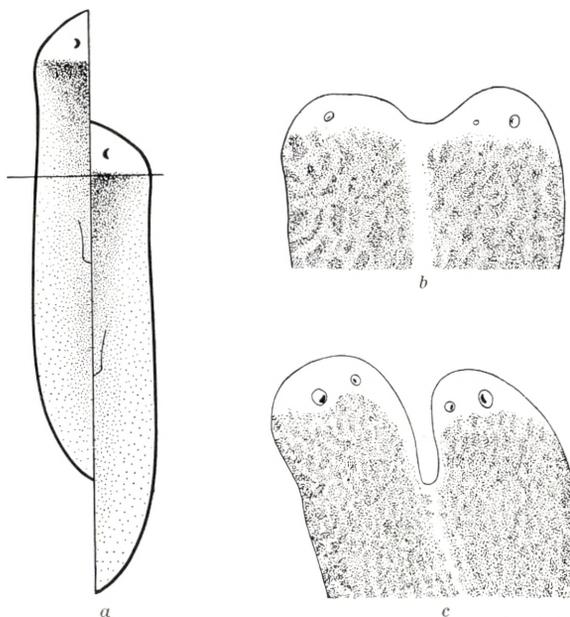


Fig. 9. *Euplanaria lugubris*. *a*. The two halves of a split animal are transplanted together with a grade of shifting of about $\frac{1}{4}$ of the body length. The stipling indicates the distribution of the time-graded field. After healing, the chimera was transected as indicated. *b*. The situation after 7 days. *c*. The situation after 15 days.

eye and one small left eye. At the same time, the left half had regenerated a small left eye spot (Fig. 9 *b*). Four days later, both halves had regenerated distinct heads with two normal eyes each. This experiment shows that the two half-fields act like two separate entities which, when placed in a position impeding them from cooperation, regenerate the missing symmetrical half. Fig. 9 *c* demonstrates the situation after 15 days. In the beginning, the blastema formed at the transverse cut I is continuous from right to left.

The other animal (Fig. 10 *a*) which had regenerated two

heads at cut I was operated, as indicated in Fig. 10 *a* II, 8 days later. Here, the field was met at a level about $\frac{1}{4}$ caudad in the right half and about $\frac{1}{2}$ caudad in the left half.

After ten days a symmetrical head had regenerated. Obviously, the two levels met by cut II are not so divergent in their rate of

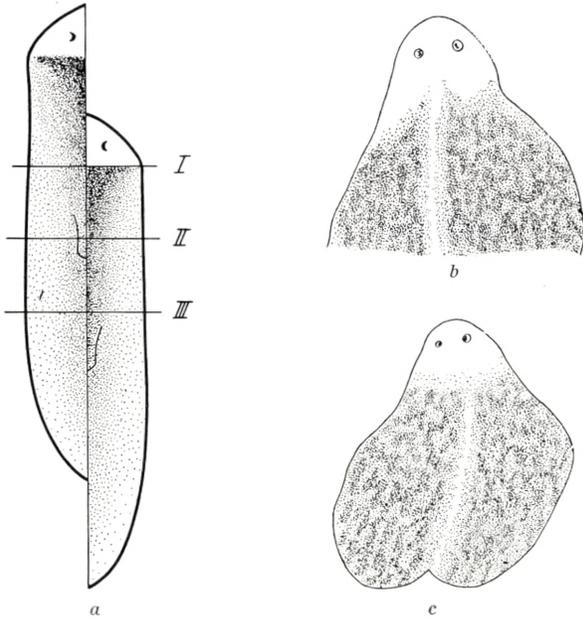


Fig. 10. *Euplanaria lugubris*. *a* indicates the experimental procedure. *b*, the regenerate after 10 days from the transverse cut II in *a*. *c*, the regenerate after 18 days from the transverse cut III in *a*.

regeneration as to prevent their cooperation. Fig. 10 *b* shows the result; the slightly larger right eye is especially noteworthy.

18 days after decapitation, the remaining hindpart was again cut transversely (Fig. 10 *a* III), the cut meeting the field in the right piece about half way down, in the left one about $\frac{3}{4}$ caudad. Fig. 10 *c* indicates that the two fields have again been able to cooperate.

Example b. The two halves were joined as shown in Fig. 11 *a*. The forepart of the left half cytolized (Fig. 11 *b*). Decapitation was performed after 72 hours. The cut met the field about $\frac{1}{4}$ down in the right half and $\frac{3}{4}$ down in the left half. Seven days later, the right half had developed one larger right eye and a

smaller left one, whereas the left half had not regenerated any eye at all. Fig. 11 *c* shows the situation after 15 days.

Example c. The two halves were joined (Fig. 12 *a*) and their foreparts separated a little bit (Fig. 12 *b*). 72 hours after transplantation a transverse cut was inserted (Fig. 12 *a*). After seven

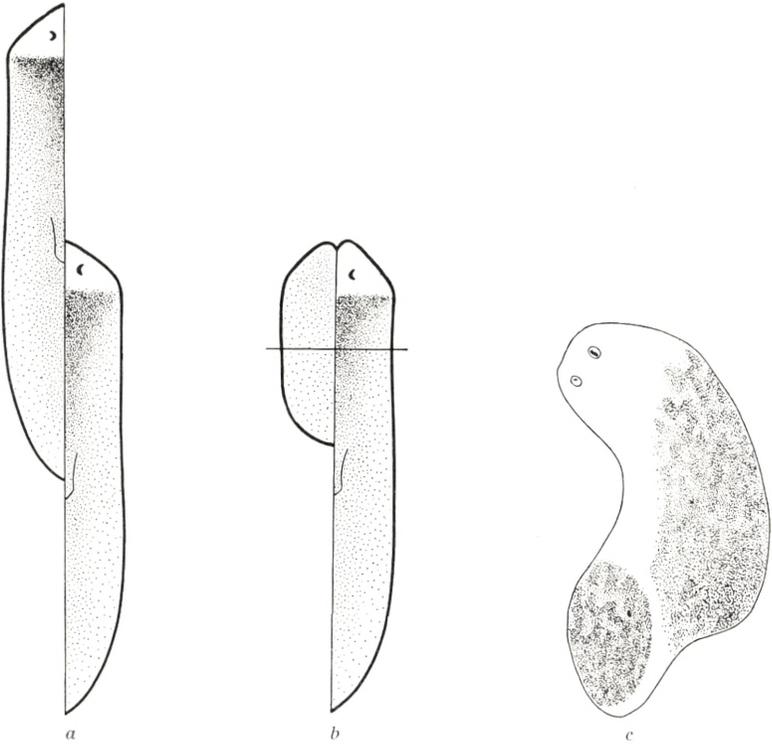


Fig. 11. *Euplanaria lugubris*. *a* shows that the lengthwise transplantation has been made with a shifting, covering about half the length of the animal. *b* shows the transection after the forepart of the left half-animal had cytolized. *c* gives the result after 15 days of regeneration, only the right half has regenerated eyes.

days, the right piece with its high regeneration rate developed one larger right eye and a smaller left one. 11 days later, the situation was unchanged, no regeneration was observable in the left piece (Fig. 12 *b*).

Example d. The two halves were transplanted as in Fig. 13 *a* (i. e. almost in the same way as specimen *a* in Fig. 9). The free anterior part of the left half cytolized and the foreparts of the two halves did not coalesce (Fig. 13 *b*). The first transverse cut

was made about $\frac{1}{4}$ down in the right half and about half way down in the left one. Seven days after decapitation, the right half had regenerated two eyes, the right one being the larger. The left half had not regenerated any eye. Fig. 13 c indicates the situation after 16 days. Subsequently, the chimera was cut again (Fig. 13 b). The right half was met about midway, the left

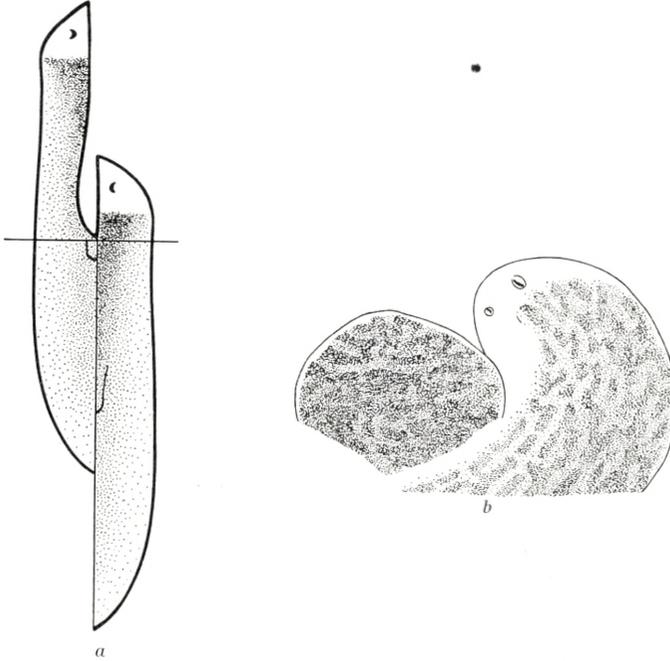


Fig. 12. *Euplanaria lugubris*. a, the experiment; b, the result which is the same as in fig. 11 in spite of the fact that the left half animal retains its integrity throughout the healing period.

one about $\frac{3}{4}$ down in the field. No cooperation had taken place; only the right half has regenerated two eyes, the right one being slightly larger than the left one (Fig. 13 d).

Example e. The two halves of four animals were transplanted; the fields were slightly shifted, about $\frac{1}{6}$ of the length of the specimen (Fig. 14 a). 72 hours after transplantation the foreparts were removed as indicated by the line. Ten days later, an almost symmetrical head had regenerated, the right eye being only slightly larger than the left one, indicating the slightly higher regeneration rate of the right field where the cut had hit (Fig. 14 b).

Example f. The two halves of three animals were transplanted with a very slight transposition, only about $\frac{1}{10}$ of the length of the animal (Fig. 15 *a*). 100 hours after transplantation a cut was made, as indicated by the line. Four days later, the common blastema had developed two symmetrical eyes, the right one being slightly larger than the left one (Fig. 15 *b*). Another trans-

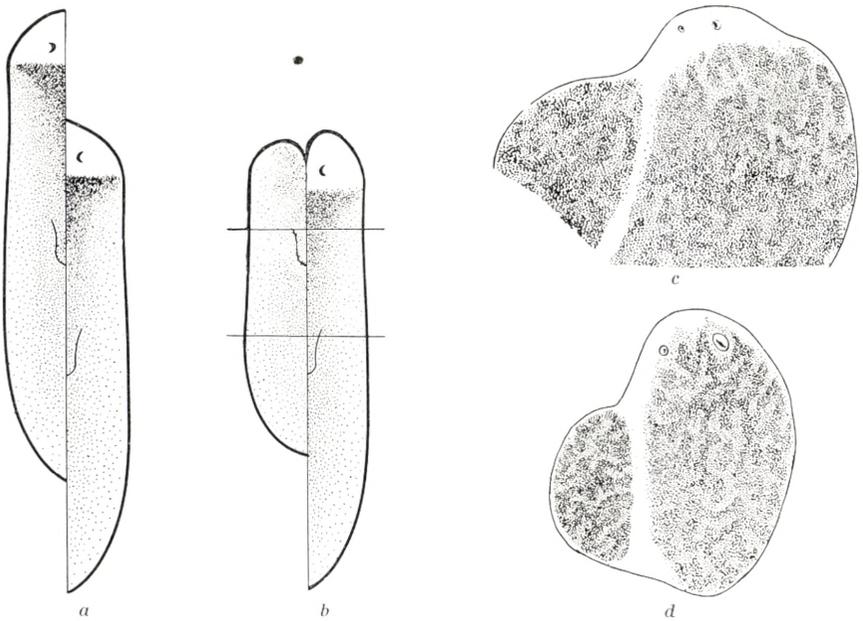


Fig. 13. *Euplanaria lugubris*. *a*, lengthwise transplantation as in Fig. 9 *a*; *b*, the anterior transverse cut was made somewhat more posteriorly than in Fig. 9; *c*, the result of this cut; *d*, the result of the more posterior cut seen in *b*; in spite of common blastemata, both *c* and *d* show eye formation only by the right half.

plantation was made at the same level and showed the same result, although no difference in the size of the eyes was detectable.

Example g. The transplantation was made in the same way as in specimen *f*; the two halves coalesced only along a small part of the hindbody (Fig. 16 *a*). 100 hours after transplantation the cut was made. Five days later, a common blastema had developed (Fig. 16 *b*); ten days later, a trace of a right eye could be observed, and four days later, two symmetrical eyes had been regenerated, the right being a trifle larger than the left one (Fig. 16 *c*).

Example h. The same transplantation as in *f*; also here the halves coalesced only slightly. A cut was made in about the same place as in *g*, but the wound contracted so much that no wound surface was exposed and, hence, no blastema formation took place. Nine days later, a new cut was made a little behind

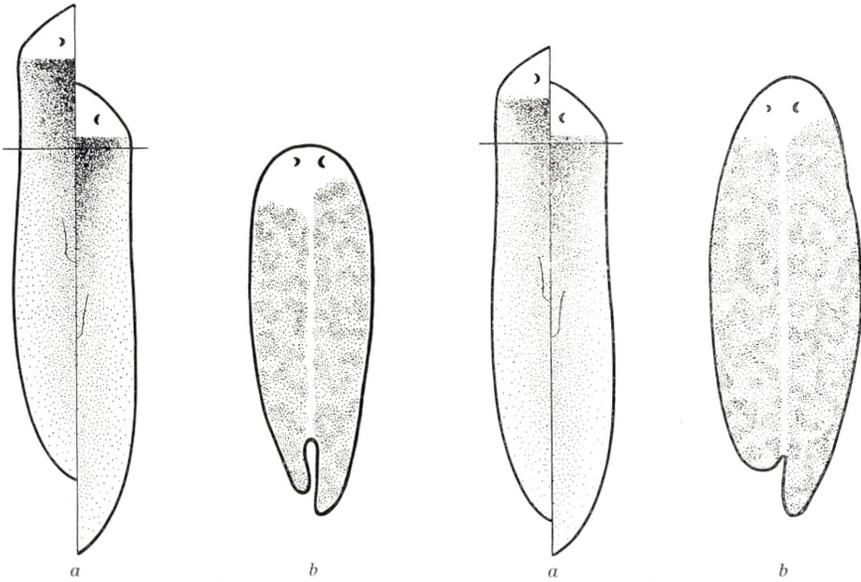


Fig. 14.

Fig. 15.

Fig. 14. *Euplanaria lugubris*. *a*, the shifting covers about $\frac{1}{6}$ of the animal's length; *b*, a bilateral symmetrical head is regenerated, the right eye, however, being slightly larger than the left one, due to the fact that the right half has been met at a level with higher regeneration rate than the left one.

Fig. 15. *Euplanaria lugubris*. *a*, shifting only about $\frac{1}{10}$ of the animal's length; *b*, the result as in Fig. 14.

the first one in order to obtain an exposed surface (Fig. 17 *a*); again the wound healed up and no blastema formation took place. 17 days later, another attempt was made by inserting two cuts (Fig. 17 *b*), but this was not successful either.

Similar transplantations were made on *Bdellocephala punctata*; only one of the operations was successful. The two halves were shifted about $\frac{1}{5}$ of the length of the animal. Six days after decapitation, a common blastema had formed one large right and one small left eye.

III. Parabioses and the Time-Graded Field.

In a previous paper (BRØNDSTED 1942), it has been shown that two longitudinal halves from different specimens, when joined and decapitated, form a common blastema with two

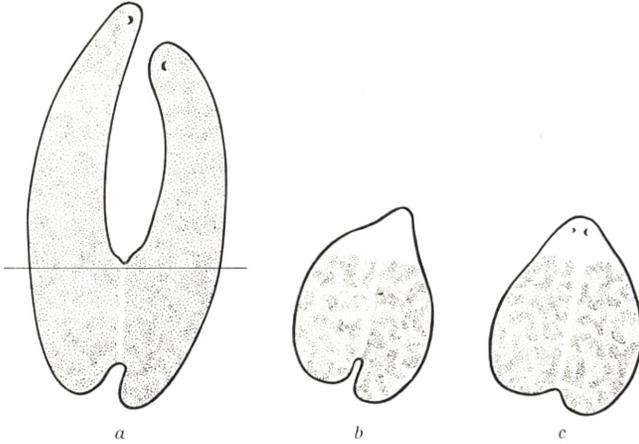


Fig. 16. *Euplanaria lugubris*. *a*, the shifting covered only about $\frac{1}{10}$ of that in Fig. 15; only the posterior third of the halves coalesced; the transverse cut was made as indicated by the line; *b*, the situation after 5 days; *c*, after 10 days; the right eye was a trifle larger than the left one; thus, also at this posterior level, the field displays itself.

symmetrical eyes. We were interested in learning the reaction of two whole animals to transplantation and subsequent decapitation.

The operation was carried out in the following way. In nicotine anesthesia, a longitudinal lateral brim was cut away on either the right or the left side of two animals, and the two pieces were united immediately.

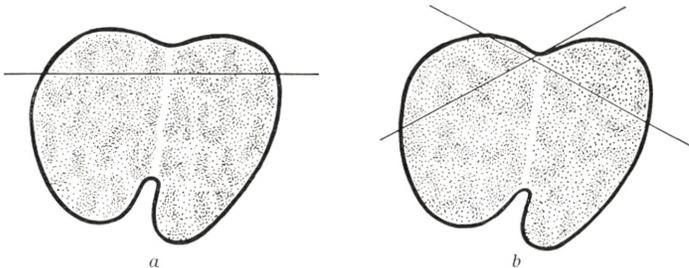


Fig. 17. *Euplanaria lugubris*. *a*, hind parts of an animal treated as in Fig. 16. A transverse cut resulted in closing up of the wound. *b*, two cuts made as indicated had the same effect, therefore no regeneration followed.

In a first series, 16 specimens of *Planaria torva* were treated as shown in Figs. 18 *a*—*b*. The fields of the two individuals were at the same level. Only one out of eight pairs survived; it was decapitated (Fig. 18 *b*) five days after transplantation. Four days later, the right "twin" had regenerated two eyes, the left one only one small eye, but four days later again, both animals had developed a head, each having two symmetrical eyes (Fig. 18 *c*).

In a second series, 6 specimens of the hardier *Euplanaria lugu-*

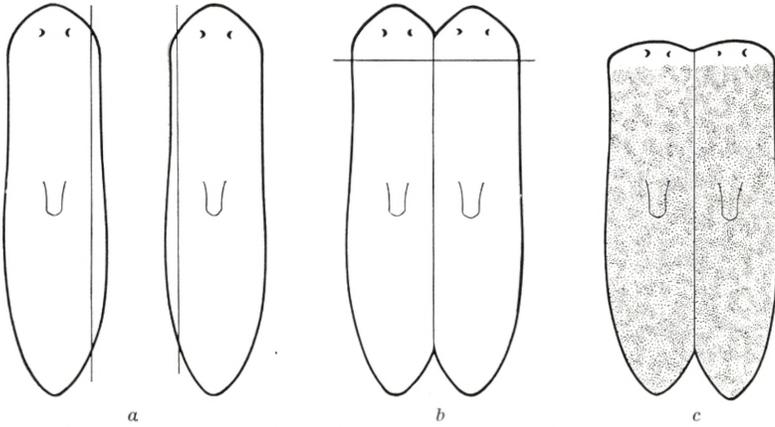


Fig. 18. *Planaria torva*. Parabioses. Narrow lateral brims were cut away from two animals, *a*; the specimens transplanted and decapitated, *b*; each animal formed its own blastema, in each of which two symmetrical eyes were regenerated, *c*.

bris were treated in the same way; they formed three pairs. The first pair was decapitated on the fourth day after transplantation. Three days later, a small blastema had developed, and six days after decapitation two symmetrical eyes in the common blastema had been regenerated, the right one being slightly larger (Fig. 19 *a*). The second pair developed in just the same way, however the two eyes did not differ (Fig. 19 *b*). On the fourth day after decapitation, each of the "twins" in the third pair had formed a blastema, although they were confluent; the right one was larger than the left (Fig. 19 *c*). In this pair, the pharynges were further apart than those in the first and second pair, indicating that narrower lateral strips had been cut away before transplantation. On the sixth day, the right animal had regenerated two eyes of equal size, whereas in the left animal no eye could be seen, and its blastema had not become larger. This situation

remained unchanged during one month, during which time the right animal had developed normally (Fig. 19 *d*), while the left animal still had no head.

The first and second pairs exhibited a continuous process of coalescence of the two animals, which appeared from the pharynges that approached the bisector more and more and almost met after a month, when they were fixed for histological examina-

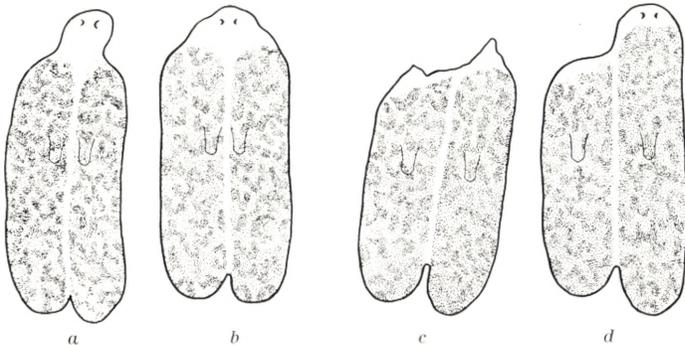


Fig. 19. *Euplanaria lugubris*. Rather broad lateral brims were cut away from four animals, which were then transplanted pairwise parabiotically. In both pairs a common blastema developed after decapitation, and in both only one head was regenerated; in *a*, the right eye was slightly larger than the left one. In *c*, more lateral brims were cut away than in Fig. 19 *a*. Hence each of the animals formed blastemata, but they were confluent. Only in the right animal a head was regenerated, *d*, presumably due to an inhibiting influence from the right to the left part of the common blastema.

tion. We are here confronted with a most fascinating phenomenon: two adult individuals merging into one by morphallactic processes. It would certainly be desirable to investigate this phenomenon in greater detail.

IV. Spreading of an Inhibitory Influence from the Median "High-Point".

The notion of a time-graded regeneration field consisting of separated points with different rates of regeneration presupposes that the high-point, exposed by the cut, acts in two ways. In the first place, it "organizes" the neoblasts into head and eye formation and, in the second place, it inhibits the formation of similar structures in other parts of the blastema, preventing them from developing their potencies for head formation.

The investigation of the subtly balanced biochemical processes involved in this phenomenon would be premature. However, suitable experiments might elucidate some details of the propagation regarding the postulated inhibitory influence from the high-point, especially with regard to time.

110 specimens of *Dendrocoelum lacteum* were cut in the way

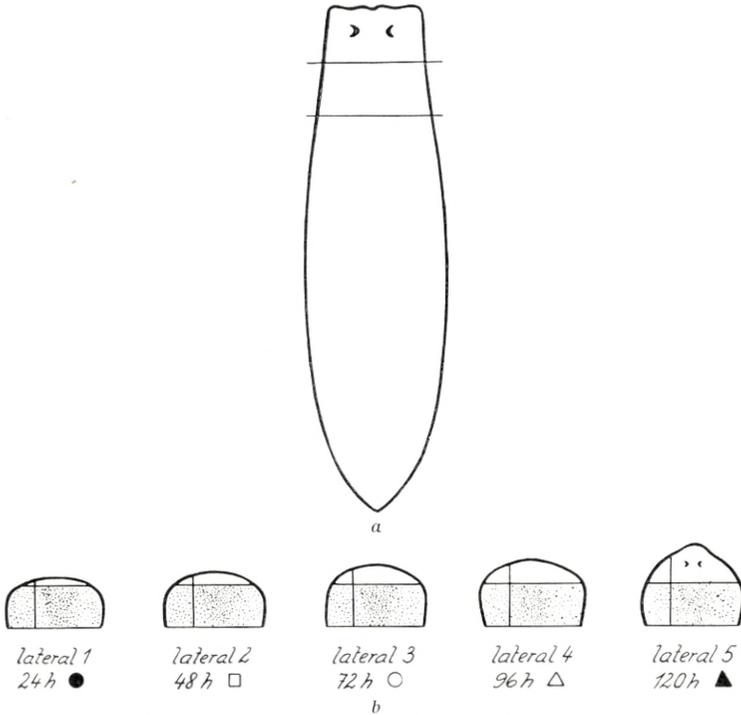


Fig. 20. *Dendrocoelum lacteum*. Representation of cuts mentioned in the text. *a*, after decapitation a transverse segment is cut out. *b*, segments with growing blastemata; the stippling indicates the time-graded field. Every 24 hours new groups of 15 individuals had their left third cut away.

indicated in Fig. 20 *a*. 10 segments served as controls, the remaining 100 segments were divided into five lots of 20 pieces each.

If an inhibitory influence from the median high-point prevented lateral parts from regenerating eyes, it should be possible, by separating the lateral parts from the median ones at different intervals, to obtain some information about the time required for the inhibitory force to travel laterally through the blastema.

The cut shown in Fig. 20 *b* separated, as sharply as pos-

sible, one lateral third of the segments from the remaining two thirds. It had to be made outside the area of normal eye-regeneration so that no tissue of the median high-point was included in the lateral third piece. On the other hand, the cut must not be made too far laterally in order to avoid too high mortality of the small pieces. Variations in the rate of regeneration cannot be completely excluded, since it is of course impossible exactly to hit the desired level of the regeneration field in all pieces; lots of 20 specimens should, however, provide a reliable mean value.

Lot 1 (Fig. 20 *b*) was cut 24 hours after decapitation, the other lots at intervals of 24 hours. The death rate of the lateral pieces was high, about 25–30 %. Several pieces bent so much towards the cut side that the wound closed completely and regeneration was inhibited.

The animals were placed in a dark room at a constant temperature of $20 \pm 0.5^\circ$ C. Regeneration was regarded as established when eyespots could be seen at a magnification of 25 in standard illumination. It should be remembered that the part of the segments which includes the median high-point regenerated two eyes, since it comprises both the left and the right parts of the bilateral field; on the other hand, the lateral parts regenerated first one eye only, e. g. that belonging to the corresponding part of the lateral field; only later is the symmetrical field built up as indicated by the regeneration of the second eyespot. In the lateral pieces, regeneration was of course considered complete when the first eyespot could be discerned (Fig. 21).

The median parts (72 survivors of 110 specimens, including 10 controls) regenerated two eyes after 170 hours, most of them (96 %) already after 140 hours. The lateral parts of lot 1 (9 survivors), lot 2 (8 survivors), and lot 3 (10 survivors) regenerated eyes at almost the same rate. Lot 4 (9 survivors) and lot 5 (10 survivors) exhibited a greatly retarded regeneration rate.

The inhibitory influence has reached the lateral piece before visible eyespots have been regenerated in the median piece. The inhibition is increased only slightly in the interval of 96–120 hours, which is about the time required for production of visible eyes in the median high-point. We shall see below that, when eyespots are regenerated, the time-graded field is restored, i. e. regeneration starts in every part of the new field at a normal rate.

Although the available material of *Planaria torva* was insufficient, an experiment was attempted on 50 animals. It would not be safe to base a hypothesis concerning the propagation of inhibitory forces on such a poor experiment, but in connection with the experiment on *Dendrocoelum* it might be permissible to regard it as a support of the hypothesis. The 50 animals available were divided into five lots and treated in exactly the same way as described above. In *Planaria torva* the median pieces regenerated two eyes at a somewhat lower rate than in *Dendrocoelum*.

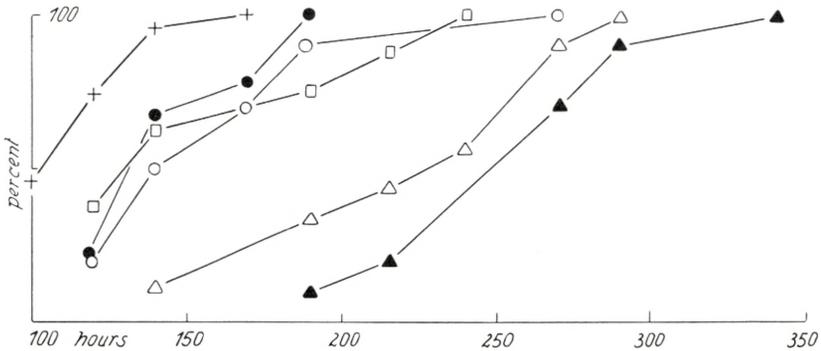


Fig. 21. Comparison with the symbols in Fig. 20 shows how the regeneration is retarded in the lateral pieces from those groups which were separated first after 96 and 120 hours.

Mortality and distortion of the lateral pieces were very great. Lot 1 (2 survivors), lot 2 (2 survivors), and lot 3 (5 survivors) also here regenerated eyes at a normal rate, whereas lots 4 and 5 (2 survivors each) were retarded.

A third experiment was carried out on *Bdellocephala punctata* in order to find out whether the result was changed if the lateral pieces were not removed from the main body during regeneration. 95 animals were decapitated at an anterior level. A lot (no. 0) of 10 specimens was cut as shown in Fig. 1 and employed as controls for the regeneration rate of "arms". This operation removes the median high-point with its inhibitory influence. 75 specimens were divided into 5 lots of 15 each (Fig. 22); they were operated upon in the same way, at 24 hours intervals after decapitation. 10 animals, which were only decapitated to serve as controls for normal median regeneration rate, had regenerated two eyes after 110 hours. It may be mentioned here that *Bdel-*

locephala, when decapitated at an anterior level, regenerates eyes at a higher rate than *Dendrocoelum*, a fact which is important for the evaluation of the results in Fig. 23.

Experiments of this type involve some difficulties. The lateral "arms", resulting from the removal of the median part, frequently detach themselves from the main body, curl up, disintegrate or close their wounds so that no regeneration occurs. In other cases, the two arms stick together and must often be separated repeatedly, a procedure which has to be performed very carefully so

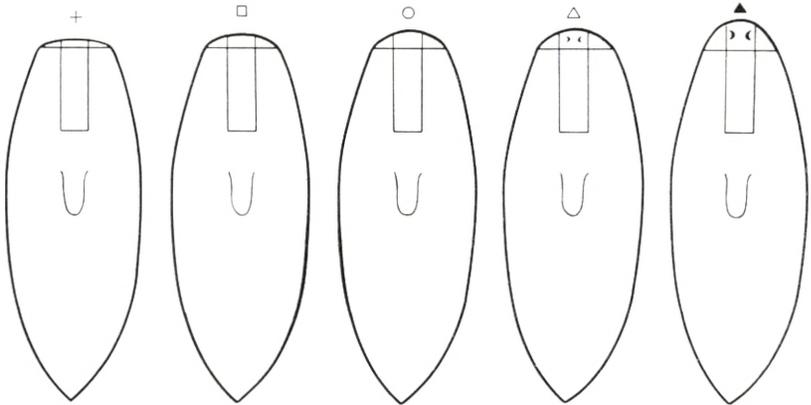


Fig. 22. *Bdellocephala punctata*. The median part was cut away after 24 hours +, 48 h. □, 72 h. ○, 96 h. △ and 120 h. ▲.

that no newly formed blastemata are removed. A fine glass needle with a little bead instead of a point proved to be a very well suited tool. Although 15 animals were operated in each lot, thus providing 30 "arms", only 48 out of 150 were useful. The percentages given in Fig. 23 refer to the number of arms. Lots 0—2 regenerated at about the same rate; lot 3 showed a somewhat retarded regeneration, while the regeneration of lot 4 was highly retarded; lot 5, however, was again but slightly retarded in regenerating. From the figures it seems reasonable to conclude that in *Bdellocephala* the inhibition from the median part reaches the lateral parts earlier than in *Dendrocoelum*; this is in agreement with the fact that eye regeneration proceeds at a higher rate in *Bdellocephala*. But how can the recovered regeneration rate in lot 5 be explained? We know that the time-graded field is restored during regeneration. Thus, in lot 5, the eyes in the

median segments have been formed already for several hours and therefore the regeneration rate of the lateral segments increases, at least nearly, to that of the normal, intact animal.

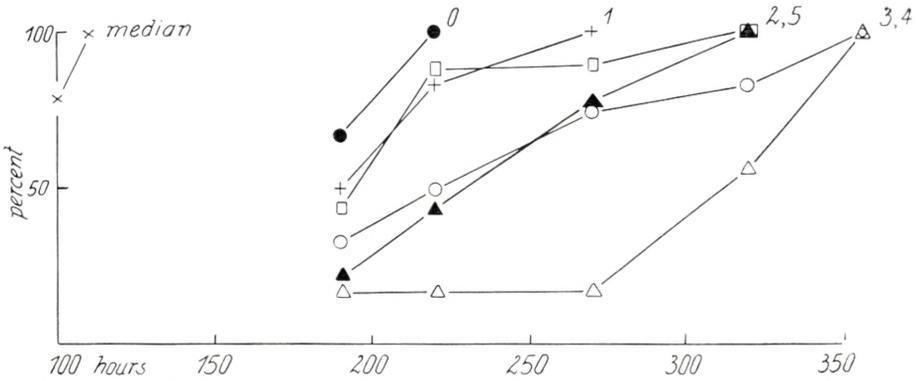


Fig. 23. *Bdellocephala punctata*. Results of the experiments represented in Fig. 22.

Furthermore, when the eyes are regenerated, the intactness of the animal is restored and only slight inhibitory forces are in action, i. e. those still present from the period when the blastema released such forces. The faster regeneration rate of lot 5 may therefore indicate that normalcy is restored in the animals after 120 hours of regeneration, as illustrated schematically in Fig. 24.

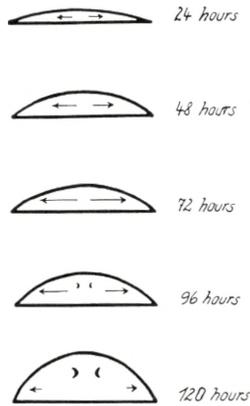


Fig. 24. Migration of inhibition in the blastema, the effect being greatest from 72—96 hours of regeneration (at an anterior level in the time-graded field), declining to zero when head formation has been fully established. The arrows indicate the direction and force of the inhibitory influence emanating from the median high-point at various times after decapitation.

**V. The Rate of Regeneration Expressed by
Differentiation is Dependent on Factors in the Time-Graded
Field; the Later Growth of Differentiated Parts is
Dependent on Available Neoblasts.**

It has earlier been shown (A. and H. V. BRØNDSTED, 1954) that the first discernible eyespots appear at the same rate in short segments and in whole decapitated animals, provided that

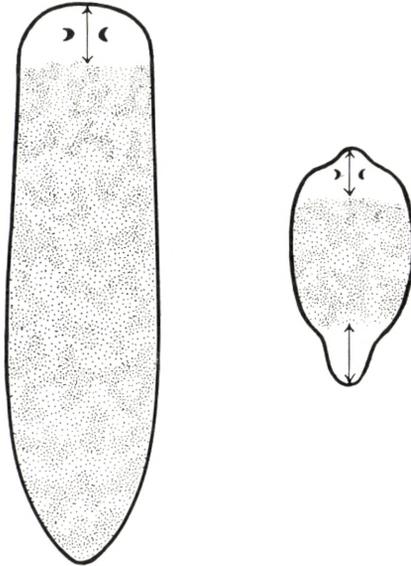


Fig. 25. *Euplanaria lugubris*. Regeneration from identical levels starts at the same time in decapitated animals and in short transverse sections; in the bigger piece with more neoblasts the bulk of regenerated parts is larger.

the cuts were inserted at the same level of the time-graded field; they appear also at the same rate even if large lateral pieces are cut away from the animal. This suggests the assumption that neither the size of a regenerating segment nor the extension of the wound is decisive for the rate of eye regeneration, the decisive factor rather being inherent in the various levels of the time-graded field. When blastema organization is terminated, the organs develop and continue to grow until they have reached a size conforming to the remnant body. This assumption has been confirmed by measurements performed on animals used in our earlier experiments (1954).

Fig. 25 shows two types of regenerating pieces from *Euplanaria lugubris*, viz. on the left side, I, the decapitated animal, on the right side, II, a short segment in which the anterior cut is inserted at the same level as in the decapitated animal. Eyespots regenerated at the same time in I and II. Four days after decapitation all eyespots could be seen, indicating that organization of the blastema was terminated. Four days later, the animals were fixed in formalin in order to enable exact measurements. At that time, the specimens in group II had regenerated short tails. We have measured the longitudinal diameters of the left and right eyespots in the 20 specimens of group I, and the distances between the anterior tip of the blastema and the foremost brim of the old pigmented part of the body. In II, also the distances between the tail tip and the hindmost brim of the pigmented part were measured.

As longitudinal diameters of the eyespots we found, in I, $23.12 \pm 0.59 \mu$, in II, $15.52 \pm 0.53 \mu$. The distance between the anterior tip of the blastema and the forebrim of the pigmented part of the body was found to be, in group I, 1.44 ± 0.04 mm, in group II, 0.96 ± 0.04 mm. The distance from the tail tip to the pigmented part was measured to 1.44 ± 0.07 mm in group II. Statistical treatment showed the significance of the differences in the values.

An experiment on *Bdellocephala punctata* was carried out in order to find out whether large wounds retard the regeneration of eyes. Fig. 26 shows that the eyes were regenerated at the same rate in animals without lateral wounds, but decapitated at the level of the time-graded field, and in animals with large wounds. The animals were fixed in formalin when all pieces had regenerated eyes. The values found are as follows. Average longitudinal diameter of the eyes in lot I (without lateral wounds) $41.9 \pm 1.82 \mu$, in lot II (with lateral wounds) $35.22 \pm 2.11 \mu$. Also this difference proved to be significant. The measurements seem to indicate that the growth of the eyes, after first differentiation, depends on the supply of neoblasts.

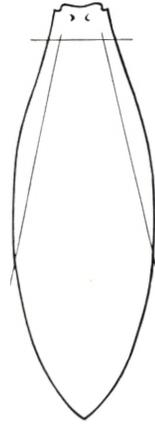


Fig. 26. *Bdellocephala punctata*. The lines indicate the cutting intending to diminish the flow of neoblasts to the transverse anterior wound (BRÖNSTED, A. and H. V., J. Embr. exp. Morphol. 2, 1954, fig. 2b.)

The difference between the values found for eye size in *Euplanaria lugubris* and in *Bdellocephala*, in groups I and II, is due to the fact that *Euplanaria lugubris* had been allowed four days of growth before fixation and measurement.

VI. Remoulding of the Time-Graded Regeneration Field during Regeneration in *Polycelis nigra*.

ABELOOS (1930) observed that, when regenerated tissue is induced (by a new cut) to undergo a second regeneration, the rate of regeneration corresponds to that of the new-formed level in the segment in which the cut was made. SIVICKIS (1933) stated that, when anterior parts of the body have accomplished regeneration and thus restored the whole animal, the rate of regeneration of the various segments of the new body corresponds closely to that in an untreated animal and not to that of the original segment from which second regeneration has started. This finding is in agreement with the results obtained by CHILD and WATANABE (1935).

Some authors seem to have regarded this phenomenon as an indication of rejuvenation of the whole animal caused by the regeneration process proper. I have also frequently observed this phenomenon in experiments on planarian regeneration. After having detected the time-graded regeneration field (BRØNDSTED 1946), I suggested that this field might be remoulded during regeneration at the same time during which observable morphological regeneration occurs.

A cut in the blastema or the regenerated tissue of course traverses embryonic or at least young tissue; hence it is plausible that repeated regeneration proceeds here at a higher rate. Now, the problem arises whether the *old parts* of the body are influenced by the regeneration process in such a way as to alter the regeneration rate at a certain level. Since it is well known that the regeneration rate is a physiological property characteristic of every part of the planarian body, we may now ask: is this physiological property of adult tissue changed by regeneration processes? A quantitative investigation is necessary before this feature of the time-graded regeneration field can be elucidated in greater detail.

Polycelis nigra was chosen for our experiments. This planarian

species is known to have a caudally declining head frequency curve, and it may be assumed that, correspondingly, the time required for head formation increases caudally.

For the regeneration of eyes at six consecutive levels of the body, SIVICKI (1931) found the following rates (in hours): 104, 124, 139, 151, 181, 175. Although our experimental animals had been collected in another locality than SIVICKI's—both in our earlier studies (BRØNDSTED 1942) and in the present investigations, we used planarians from the Furesø, a lake in the vicinity of Copenhagen—the head frequency curves are largely similar.

Despite the fact that, in 1942, I had not yet been fully aware of the existence of a time-graded regeneration field in planarians, I was able to show that the regeneration along the main axis of the forepart of the body of *Polycelis nigra* follows the laws of such a field, presumably on almost the same lines as that of *Euplanaria lugubris* (A. and H. V. BRØNDSTED, 1953).

So far, the exact shape of the time-graded regeneration field of *Polycelis nigra* has not yet been worked out. However, SIVICKI's results, as well as my earlier experiments (BRØNDSTED 1942) and the findings of my control series 0 and I in the present paper, indicate that the time required for eye regeneration after a transverse cut increases the more, the more caudally the cut is inserted. This makes it probable that an antero-caudal axis of a time-graded regeneration field declining caudally does exist in *Polycelis nigra*; for the present investigations, it is not necessary to know the lateral extension of this field.

We have chosen *Polycelis* not only because this species possesses an antero-caudal time-graded regeneration field, but in addition because the great number of eyes in this species is convenient for estimating the degree of regeneration, and the position of the eyestrings makes it possible to perform sectioning rather accurately at a certain level.

Method.

115 specimens were cut transversely just behind the eyestring, as indicated by *a* in Fig. 27. Fifteen were kept for regeneration (control series 0), fifteen were immediately cut again at the level indicated by *b*, about 0.5 mm behind cut *a* and anterior to the

pharynx (control series I). The animals in the experimental groups were cut a second time at the same level as control series I, but at varying times after the initial operation, viz. 2 days (group I), 4 days (group II), 6 days (group III), 7 days (group IV), and 8 days (group V).

The aim of these experiments was to find whether, after removal of the regenerated part at a certain level of the old tissues at different periods after the first cut, regeneration started earlier from the new cut than in control series I in which the cut had been inserted at the same level. If this were true, a clear proof had been provided for the remoulding of the time-graded field in its antero-caudal axis.

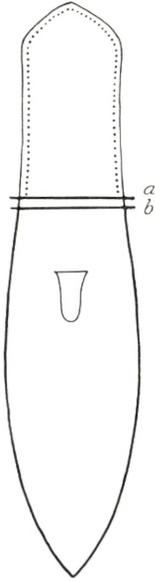


Fig. 27. *Polycelis nigra*. Decapitation *a*. Cutting away the forthcoming blastemata at *b* after varying times.

The experiments involve certain difficulties, insofar as no eye regeneration takes place if the transverse cut is closed by a strong contraction of the body. If the wound closes partially, eye regeneration proceeds at a slightly lower rate than with an open wound. For the estimation of the regeneration rate, animals with closed cuts had to be discarded. A further difficulty arises from the movements of the worms, which make it hard to insert the cut at exactly the desired place. Narcotization, to prevent movements, in general causes a strong contraction. If the worms are placed in very little water and

put on a wax plate, a fair degree of exactitude can be reached in the operation. Nevertheless, slight deviations from the desired level cannot be avoided, differences in the level appearing as differences in the time required for eye regeneration. Therefore, a certain minimum number of animals in each group—at least 10 to 15—must be operated in order to secure a reliable mean value of the interval between the operation and the appearance of the eyes. Since counting of the eyes is very time-consuming, the handling of larger groups is not feasible.

The eyes were counted by means of a binocular dissecting microscope, magnification $25\times$, with standard illumination; light

from above proved to be most suitable for providing reliable counts. The animals were kept in Petri dishes filled with 50 ml tap water at a temperature of $20^{\circ}\text{C} \pm 0.5^{\circ}$; during the experiment, the animals in the Petri dishes were placed on white paper as a background and kept in the dark during regeneration.

The number of eyes formed in each specimen was counted daily. For each day, the total number of eyes in each group was divided by the number of living animals, giving a mean value of the number of eyes regenerated per animal.

Results.

The curves drawn in Fig. 28 show our results. The ordinates represent the average number of eyes per animal, the abscissae the time (in hours) elapsed until their regeneration.

In spite of the irregularity of the curves, they exhibit one main feature, viz. the new-formation in the adult tissues of the antero-caudal axis of the time-graded regeneration field during regeneration. The curve for control series 0 represents the regeneration rate of eyes at a transverse level just behind the eyestring; the curve of control series I at a level a little more caudally. Curves I—V show that the time required for the regeneration of a certain number of eyes decreased by about 80 hours from

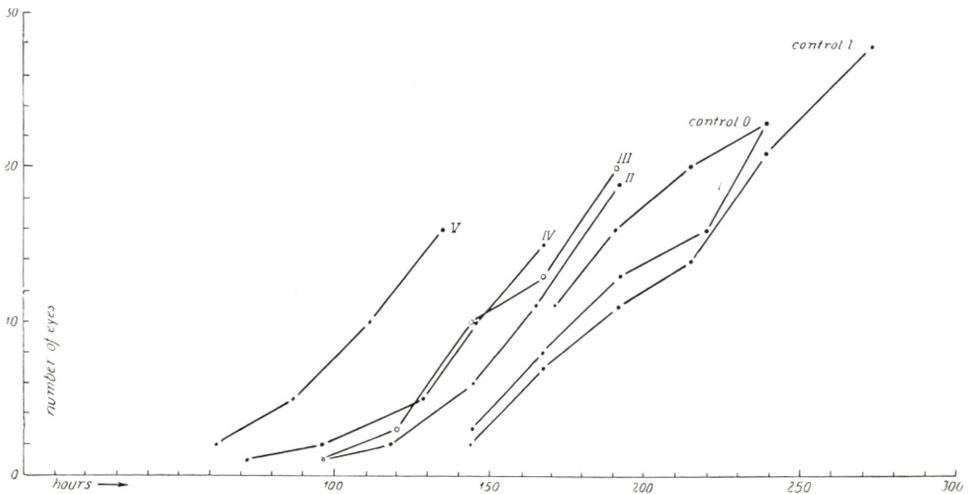


Fig. 28. *Polycelis nigra*. Eye formation in various groups. Explanation in the text.

control series I to the experimental group V. If no change in the antero-caudal axis of a time-graded regeneration field had occurred in the original tissue, eye formation should proceed at the same rate in the five groups as in control group I, e. g. in the course of 220 hours. However, our experiments show that when a new cut *b* is made after 8 days regeneration from a cut *a*, level *b* requires only 135 hours to regenerate the same number of eyes as had regenerated by level *b* in control I after 220 hours. It is therefore obvious that not only does a morphologically discernible regeneration occur from cut *a* of the removed part, but in the original tissues also a process of remoulding takes place of the structures and functions underlying the phenomenon of the antero-caudal axis of the time-graded regeneration field. In the course of 2—8 days regeneration in the various groups, level *b* has adapted its rate of regeneration to that of a far more anterior level; during regeneration of a forepart, the morphological level *b* acquires the physiological properties characteristic of more anterior levels. This indicates that the morphological level *b* is physiologically adapted to the much shortened main axis and, thus, the main axis acquires its property of being evenly time-graded.

In another investigation performed on *Bdellocephala punctata*, it could be shown that six animals which had been cut transversely, rather caudally in the time-graded field, had developed eyes in the blastemata only 11 days later. When a new transverse cut separated the blastemata with the newly formed eyes, already after five days eyes could be seen in the new blastemata of 5 of the 6 animals. This fact indicates that the time-graded field was reconstructed during restitution of the head by the blastemata.

VII. Discussion.

All our experiments indicate that time is the decisive factor in morphogenetic processes. The adult planarian is provided with a finely balanced trigger mechanism just like a ripe oocyte not yet activated. Activation is first set to work when a piece of the body is removed—just as embryogenesis in an egg starts when fertilization has occurred.

From the papers by WOLFF and DUBOIS (1948) and DUBOIS (1949) it is known that the wound calls forth a migration of neoblasts to the wound place. The chemical mechanism involved in this migration is however unknown so far. NEEDHAM (1952) has discussed some factors which possibly are responsible for this process. In a marvellous way, the neoblasts building up the blastema participate in an organization process which in due time not only restores the missing parts, but by means of morphallaxis transforms some of the size of the adult tissues to such a degree that harmonious proportions of the animal are recovered in agreement with its reduced size. Thus, the adult planarian body may be conceived as an analogue to an egg of the regulation type, however possessing one advantage over the latter: whereas the egg in the course of time crystallizes into an organism losing more and more of its regulatory capacity, the planarian body retains this capacity.

In the study of planarian regeneration, we are thus dealing with highly embryonic organisms, in spite of the fact that we are concerned with adult animals. The neoblasts in the planarian body correspond in a certain way to the blastomeres in the blastula, with the difference, however, that in the blastula some of the blastomeres themselves are the determinators, whereas in the planarian body the totipotent neoblasts are determined by the adult tissues, at least in their main polarity; they must adapt to the existing body parts.

The elucidation of this phenomenon must be reserved to distant biochemical studies. The established fact of the time-graded field may nevertheless be a first step towards this goal.

The time-graded field is a physiological property firmly inherent in some fixed structure of the body, a fact which seems to be proved definitely by our transplantation experiments (cf. Section I). This property is specific of the species and is restored during regeneration according to the principles of restoration (regeneration) valid for all other species-specific characteristics of the animal; this seems to be proved by the experiments discussed in Section VI. No explanation of this phenomenon is available at the present time; the assumption may be put forward that the restitution of the relative number of neoblasts is responsible. For future regeneration, the restoration of the field is im-

perative, since a harmonious regeneration is secured just by the properties of the field.

This interpretation of the results recorded in Section VI appears to me much more plausible on the basis of our actual knowledge about planarian regeneration than a hypothesis of rejuvenescence of the regenerating animal.

When forming the blastema, the neoblasts start as totipotent cells, e. g. their capacity of regenerating a head is the same. In the ensuing competition, it is the time-graded field which decides the result. We still do not know its mechanism, but we assume that the activities in the area of this field are the more lively, the more densely the neoblasts are packed; this means, the earlier will the differentiation processes start. However, this fact alone cannot explain the finding that other parts of the body might differentiate in just the same way, although at a lower rate. An inhibitory action emanating from the most vigorously differentiating part must be assumed. The existence of such inhibitory forces is, to my view, proved by the experiments discussed in Section III.

In broad outline, we now realize the mechanism which secures a harmonious regeneration, although the differentiation processes themselves cannot yet be understood. A future comparison of the biochemical processes in the gastrula with those in the blastema may provide further evidence. As a first approach, the following hypothesis is suggested.

We know that the prospective ventral ectoderm in the amphibian gastrula can be induced to form neural tissue: it has neural potencies. Why are these potencies not developed in the normal gastrula? In the normal gastrula, inhibitory influences emanate from the prospective neural area in step with the determination and differentiation of the nervous system. Just the same mechanism—a time-graded morphogenetic field—is exhibited by the amphibian egg as by the planarian blastema. To my view, the formation of the axis in the oocytes is in fact a building up of the time-graded field.

In the planarian body, the time-graded field conveys its properties, so-to-say, to the blastema. Therefore, both in the egg-blastula-gastrula and in the blastema a high-point starts morphogenesis by organizing *and* inhibiting.

Another analogy may be mentioned: Ribonucleic acid (RNA) plays an important part in morphogenesis. In the amphibian egg, the high-points (dorsal parts) are characterized by a higher content of RNA. In the blastema, the neoblasts are charged with RNA (own investigations, unpublished) and it could be shown (A. and H. V. BRØNDSTED, 1953) that RNA accelerates regeneration in planarians.

The concept of the time-graded field may also lend some new perspective to the problem of bilaterality. Two symmetrical halves of a bilateral organism react in fact like two animals working harmoniously together. Only if they check each other's latent ability to produce their own mirror image, they are able to do so. A latent inhibition is thus continuously at work. Experiments have been performed in order to support this assumption. When separating an animal into two halves, each half will regenerate its symmetrical half. If, however, after separation the two halves are reunited in their former position, no regeneration occurs. If two reunited animals are decapitated, both halves develop a confluent anterior blastema in which each half regenerates an eye belonging to its own side, in spite of the fact that each half blastema has the power to regenerate a symmetrical eye, and irrespective of the amount of old tissues transplanted together (Fig. 29; BRØNDSTED 1942). A cooperation between the forces built up in the symmetrical parts of the common blastema is possible only if their rates of regeneration are not too different (cf. Section II). This finding again underlines the vital importance of the time-graded field. Theoretically, the reaction of the two time-graded fields may occur in three different ways. (1) The two fields are shifted very slightly so that one of the fields, that with the highest rate of inhibitory forces, did not have time enough to inhibit the regeneration of the other half's eye. If both halves have started in due time to inhibit each other's ability to develop a symmetrical eye of its own, a bilaterally symmetrical head is regenerated. The eye of that half which started regeneration somewhat earlier is of course somewhat larger in the beginning; later, however, full harmony is restored. Thus, the two fields are still able to cooperate. (2) The two halves have been shifted very severely so that the rate of regeneration in one of them is much higher than in the other. Then, its half

blastema will have time enough to regenerate not only its own eye, but also a new symmetrical one belonging to its own system. Simultaneously, the symmetrical part of the blastema is prevented from regenerating eyes (cf. Section III). Time is the factor determining this process. Only if the "weak" part of the blastema is so slow in regenerating that inhibitory forces are activated before it starts its own independent regeneration, the two fields cannot cooperate. (3) The fields are shifted so slightly that both halves of the common blastema, because of their sufficient regeneration rate, start redeveloping their own eyes. Actually, inhibitory forces emanate from both fields. If the shift, however, is made in such a way that the inhibitory forces from one half of the blastema are not allowed to reach the other half *before* the latter has started regenerating its own symmetrical eye, two heads will be regenerated.

These three theoretical conditions are corroborated by experiments. According to CHANDEBOIS (1950, 1952, 1953), a time-graded regeneration field exists also in marine Polyclads. She states that, in her experiments, regeneration proceeded after partial cytolysis in hypertonic seawater, resulting in a certain independence in the regeneration rate of the symmetrical halves. Her results are in good agreement with our above interpretation.

It would be interesting to investigate whether a time-graded field can also be observed in other organisms. This could be done by experimenting on a blastula or a young amphibian gastrula and, in a somewhat similar way, on chick blastoderm in its early stage. In these higher organisms, the time-graded fields may be so complicated that spectacular results might be expected.

The notion of time-graded fields differs, in my opinion, from the usual notion of morphogenetic fields in that the emphasis of morphogenetic differences is laid on the time factor in morphogenesis, although I am, of course, quite aware of the fact that the time factor has been drawn into consideration both in the comparison between, e. g., eggs of the regulation and mosaic type, and the formation and crystallization of a specific morphogenetic field. Our concept of the time-graded regeneration field, however, includes immanently the phenomenon of inhibition towards neighbouring fields which have the same possibilities, but lower rates of their realization.

In the beginning of this century, two eminent scientists, working with planarian regeneration, arrived at very diverging conclusions concerning morphogenesis. On the one hand, MORGAN held the view that proper regeneration is due to structural (qualitative) differences; CHILD, on the other hand, postulated that regeneration is determined by quantitative metabolic processes, a hypothesis which he extended to a comprehensive principle covering all morphogenesis: his well known gradient hypothesis.

To me it appears improbable that pure quantitatively graded metabolic processes should produce qualitative differences, such as those observed in all morphogenesis. Structural differences must first be created in the blastema, where they are given as *possibilities* immanent in every cell. In other, somewhat old-fashioned words, we may say: the differences are *performed*, they possess a sum of reactive possibilities, hence a structure based on inheritance. So far I agree with MORGAN. But I think that the structural possibilities ought to be set to work by some kind of quantitatively graded influences, possibly by different levels of metabolic activities, according to CHILD's postulate. The concept of the time-graded field may be useful in providing means for a reconciliation of MORGAN's with CHILD's views.

In his brilliant studies on planarian regeneration, CHILD arrived at the conclusion that the head dominates and organizes. I was able to show that CHILD's opinion is not right (BRØNDSTED, 1939, 1942). It is true that it is a head which appears first in the blastema on a cranial wound, and analogously it is a tail which first appears at a caudal blastema. The bilateral time-graded field determines only the rate at which regeneration sets in. This evidence induced CHILD to the formulation of his hypothesis. Morphogenesis proper, however, is determined by quite other forces of much more subtle nature, which are intimately connected with molecular structures and functions and are independent of the metabolic level. These are the forces which led MORGAN to the statement of his views.

The elucidation of the molecular structures involved in the processes mentioned above is a very distant goal; it is conceivable, however, that a concept of the coarser material basis of the time-graded field may be found. To this purpose, two factors

present themselves immediately to consideration, viz. (1) the amount of neoblasts, and (2) the structure of the nervous system.

CURTIS and SCHULZE (1934) advanced the view that the smaller regeneration power in *Procotylon* and *Dendrocoelum* (lack of head regeneration from cranial wounds at levels behind the anterior $\frac{1}{3}$ and to $\frac{2}{5}$ of the body) is due to the smaller number of neoblasts in the body of these species compared to those with head regeneration powers throughout the body. The number given is not convincing to me. It is true that heads are not regenerated by posterior levels at cranial wounds, while tails are newly formed at the same levels from caudal wounds. Thus, it is plausible that not only neoblasts, but also other forces must be lacking in the hind parts. Moreover, the ratio of neoblasts in *Procotylon* and *Planaria* is given to 1:8 and, nevertheless, heads from anterior levels are regenerated at almost the same rate.

These incongruencies induced us to reinvestigate the problem. Thorough countings of neoblasts stained by pyronin-methyl green, toluidin blue, etc. were carried out in our laboratory. The results of these countings (shortly to be published) do not show any significant differences in the number of neoblasts in species with greatly varying time-graded regeneration fields.

The nervous system in planarians consists primarily of symmetrical head ganglia which give off nerve trunks running longitudinally from head to tail and are connected by transverse commissures. It might well be conceived that this system is responsible for the time-graded field. Against this view it might be objected that the time-graded field is not conform with the amount of nervous tissue, and the question may be raised why head regeneration should be restricted to the forepart of the body, despite the fact that the nervous system extends over the whole body.

In brilliant studies WOLFF and LENDER (1950) and LENDER (1951, 1952, 1954) were able to show that in *Polycelis* eye formation is dependent on the presence of head ganglia. But this does not solve the problem, it only shifts it from eyes to ganglia. The head ganglia must be regenerated, at least their rudiments must be differentiated before eye formation can start. Thus, we revert to our earlier statement that the rate of morphogenesis is dependent on some factor in the time-graded field. And again MORGAN'S

view suggests itself: we have to deal with some hidden morphogenetic structure.

The question of polarity in connection with the time-graded field has been mentioned in my review of planarian regeneration (BRØNDSTED 1955). A more comprehensive discussion has to be postponed until the investigations in progress in our laboratory will be concluded.

Finally, the inhibition phenomenon may be touched upon (cf. Section III). Although the time required by the inhibitory force to traverse the body from the high-point to the lateral parts is roughly known, we are quite ignorant of the nature of these forces.

We know that these forces do not move freely through adult tissues; if they did, supernumerary heads could not be regenerated from other parts of an adult worm with an intact head (cf. BRØNDSTED 1939, 1942). The forces migrate only through embryonic tissue, the blastema. Thus, they only manifest themselves while organization is in progress. Therefore, I assume that they should be regarded as chemical stimulants in contact with one another from cell to cell. When a cell (*a*), responding to a given stimulus, is in the process of determination in a certain direction, it assumes biochemical properties of a very subtle serological kind; consequently, it becomes refractive towards its neighbouring cells (*b*) which may have received the same stimulus. Since, however, cell (*a*) has started first, it is also the first to gain the serological properties mentioned and, thus, it prevents its neighbouring cells (*b*) from acquiring exactly the same serological properties; these cells can therefore only reach differentiations of a "lower" kind.

This crude sketch is meant to indicate a possible way of approaching the inhibition problem. Elsewhere (BRØNDSTED 1954) it was suggested that the notion of the time-graded field may provide evidence for a reconciliation of the genetic totipotency of the cells with their diversification during morphogenesis.

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- LENDER, TH. (1956). Recherches expérimentales sur la nature et les propriétés de l'inducteur de la régénération des yeux de la planaire *Polycelis nigra*. J. Embr. Exp. Morphol. **4**, 196—216.
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