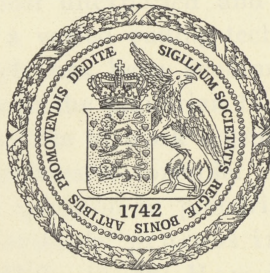


DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XX, Nr. 4

THE EXISTENCE OF
A STATIC, POTENTIAL AND GRADED
REGENERATION FIELD IN
PLANARIANS

BY

H. V. BRØNSTED



KØBENHAVN

I KOMMISSION HOS EJNAR MUNKSGAARD

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I.

An enigma in regeneration, in fact, *the* enigma, is this: which way does the adult organism restore the "wholeness" of the body, when pieces thereof have been removed?

This cardinal question can only be adequately answered when we are able fully to understand the behaviour and action of the single cell and the cooperation of all the cells concerned in the rebuilding processes.

Before this remote goal may be attained we must try to divide the regeneration enigma into minor problems which may be attacked separately.

It is of the utmost importance for success that we formulate and delimit our questions clearly and purposely.

The exquisite regeneration power of the triclad planarians has since long attracted the curiosity of several prominent biologists. It may be said—somewhat paradoxically—that a major difficulty in the interpretation of the results is the almost total and overwhelming regeneration power of some planarians. It is possible to get the most unexpected and curious regeneration phenomena by using a varied cutting technique. Hereby a bewildering mass of facts has been accumulated concerning regeneration possibilities in the planarian body. Bewildering in so far as it seemed impossible to work together the facts unto one reconciling and comprehensive formula.

Therefore the first question in planarian regeneration must be this: How is an orderly process in the regeneration of lost parts established?, or: How is the harmonious body form restored?

For some years (1936, 39, 42 a, b, 43) I have been occupied in trying to find a path through the jungle of problems in plan-

arian regeneration, and I have arrived at formulating the first problem to be resolved thus:

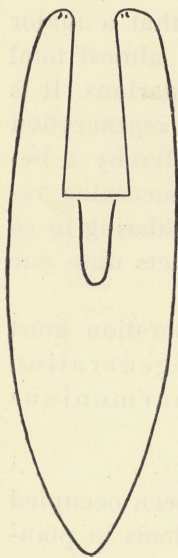
1. It is known that almost every part of the planarian body is able to regenerate a head when the old one with smaller or bigger parts of the body is cut away.

2. It is known that two or more heads may be regenerated when the cuts are made in certain definite ways (figs. 1, 2, 3).

3. Hence, therefore, why do the planarians ordinarily only regenerate one head from the surface of the wound in face of the fact that almost every point on the wound surface is able to regenerate a head?

If this problem can be solved we should have the answer to the first question in regeneration: How is an orderly process in the regeneration of lost parts established? How is the harmonious body form restored?

For several reasons it was deemed most practical to use a planarian species the regeneration power of which is not complete. Such an animal is *Bdellocephala punctata*. Most of my experiments have therefore been made on this species, which in addition has the advantages of being hardy, rather slow in movements, and rather large. In the course of my investigations other species were occasionally used to harden the facts and theories; in this paper the facts are represented on the basis of researches on *Dendrocoelum lacteum*.



II.

In the triclad planarians there exists a phenomenon, called by its discoverer CHILD, head-frequency (1921). The phenomenon is made clear by SIVICKIS (1930) in a rather complete manner.

Fig. 1. *Bdellocephala punctata*. The head and the median part of the body is cut away. Heads are regenerated at the anterior edge of the two lateral "arms".

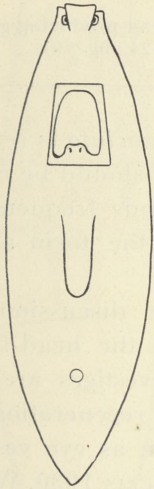


Fig. 2. *Bdellocephala punctata*. A head is regenerated in a "window" cut in the forepart of the body. The old head does not inhibit the formation of a new one. (BRØNDSTED 1942 a, p. 20, fig. 15).

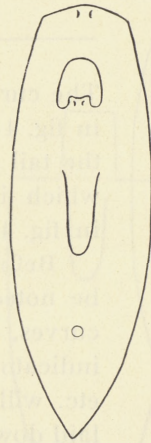


Fig. 3. *Bdellocephala punctata*. As fig. 2. The old head is cut away, instead a new head is regenerated, which does not inhibit the formation of the head in the "window". (BRØNDSTED 1942 a, p. 21, fig. 17).

Brifley stated the phenomenon is this: the ability to regenerate a head is most strongly developed just behind the eye region in the forepart of the body. From here the ability tapers caudad, in some species almost imperceptibly, in some more marked, and in others again the ability is extinguished somewhere between head and tail. In some species, where natural fission occurs, the ability, after tapering along the main axis of the first zooid, again rises in intensity in the potential head region of the next zooid.

This ability may be plotted as a curve, the head-frequency curve.

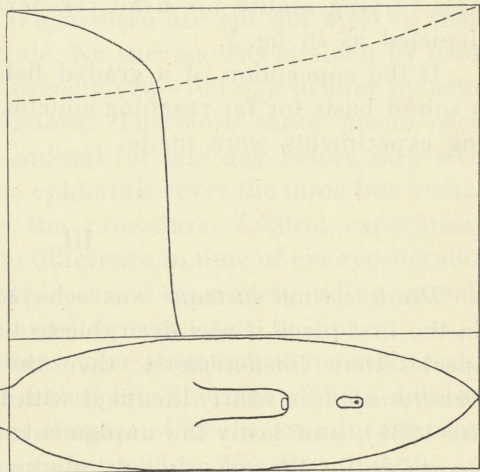


Fig. 4. *Bdellocephala punctata*. The head-frequency curve (BRØNDSTED 1939, p. 23, fig. 12).

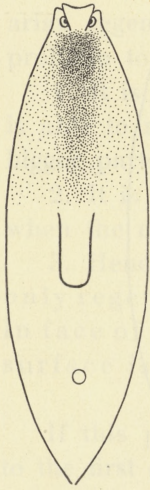


Fig. 5. *Bdellocephala punctata*. The head-producing graded field. (BRØNDSTED 1942 a. p. 24, fig. 19).

The curve for *Bdellocephala punctata* is reproduced in fig. 4 (BRØNDSTED 1939). It should be noted that the tail region has an analogous frequency-curve, which in *Bdellocephala* has the form also given in fig. 4.

Before we proceed in our discussion it must be noted that in working out the head-frequency curves, the forthcoming eye vestiges are used as indicators for the fact that full regeneration of head etc. will surely follow, as soon as eye vestiges are laid down (ABELOOS 1930, SIVICKIS 1930, WATANABE 1935, BRØNDSTED 1939).

Now, in 1942 I made some experiments suggesting that this notion of a head-frequency curve along the main axis of the animal must be extended so as to cover the demands of certain experimental results. It was made probable that the ability to regenerate heads in *Bdellocephala* not only decreases caudad but also from the middle line of the body towards both sides. If this is true, then the head-frequency phenomenon is not restricted to one dimension, the antero-posterior axis of the animal, but it would cover an area, so constituting a veritable graded field. The varying ability for head regeneration in this field may be depicted as in fig. 5.

If the conception of a graded field is right it will constitute a sound basis for far reaching conclusions. Therefore the following experiments were made.

III.

Dendrocoelum lacteum was selected as experimental animal. In the first place it was desirable to test the question on another species than *Bdellocephala*; then the head-frequency curve of *Dendrocoelum* is nearly identical with that of *Bdellocephala* (SIVICKIS 1931), and lastly the unpigmented body of *Dendrocoelum* is favorable for the experiments planned.

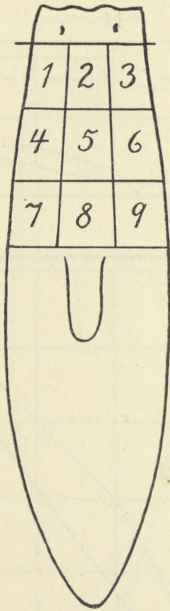


Fig. 6. *Dendrocoelum lacteum*. The forepart of the animal is cut into 9 pieces after removal of the head.

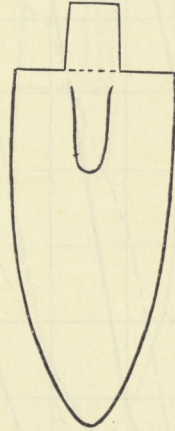


Fig. 7. *Dendrocoelum lacteum*. An "8-piece" left on the animal for one day after removal of the other pieces.

Two series, each of 15 animals, were cut as indicated in fig. 6. The 15 1-pieces were allowed to regenerate in a Petri-dish, the 15 2-pieces in another, and so on. The amount of water was identical in each dish, and so was temperature and light.

It proved necessary to use a special trick with regard to middle-pieces 2,5 and 8. When these are cut out most of them soon cytolysed and disintegrated. No success was attained by using Ringer solutions of varied strength (20—100 %), neither by using CaCl_2 in varied concentrations. Therefore these pieces were allowed to remain on the animal for one day before they were cut away, in order to let the epidermis cover the three free wound surfaces. Fig. 7 illustrates the procedure. Control experiments proved that there existed no difference in time of eye regeneration between such free 2,5 and 8 pieces and the corresponding animals, where the pieces were allowed to regenerate in situ.

Once every day the pieces were investigated for regenerated eyes. The piece was laid on a waxplate, illuminated by a standard electric lamp of 37 watt, at a distance of 20 cm, examined through a dissecting microscope magnifying 20x. The first time the eye

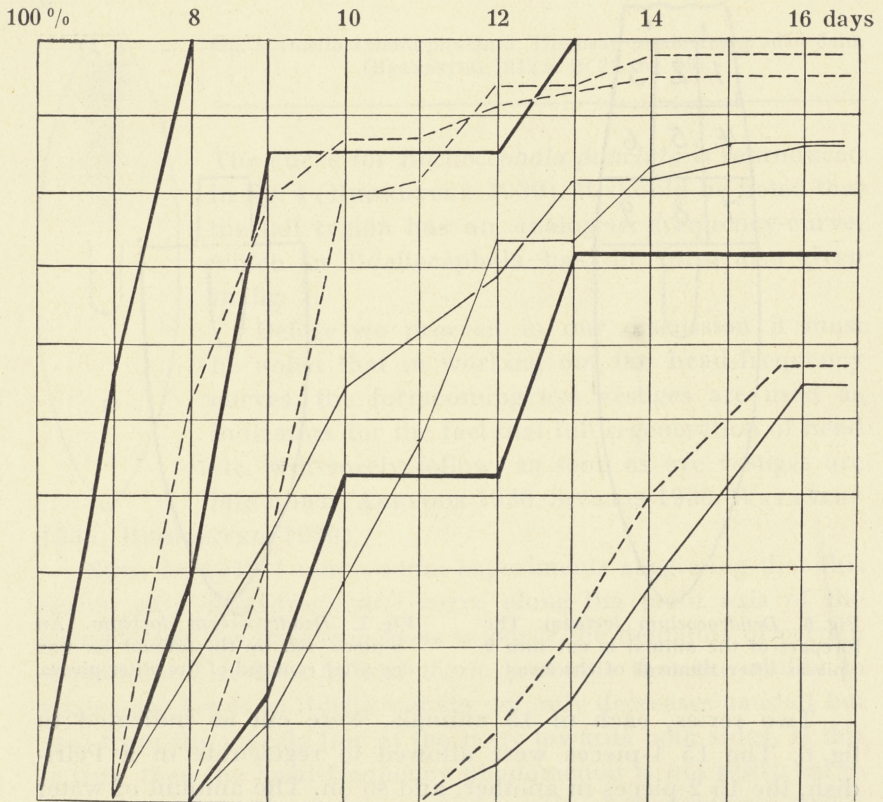


Fig. 8. *Dendrocoelum lacteum*. Graph showing regeneration power of the 9 pieces cut as in fig. 6. Explanation in text.

could be observed as a pigment spot was noted; the time was also noted when two pigment spots were regenerated. The results from the two series were much alike, therefore they were handled together in calculating out the numerical results given in fig. 8.

The ordinate of the graph fig. 8 gives percentage of pieces having regenerated eyes in so and so many days (represented by the abscissa). The three thick unbroken lines give the regeneration power (expressed in eye-formation) of piece 2, 5 and 8. These median pieces which comprise both left and right parts of the body always regenerate two eyes simultaneously. The regeneration power of these pieces coincided well with that of whole transverse sections, which have been employed to work out the head-frequency curve of *Dendrocoelum* (Sivickis 1931). This was of course to be expected, since the eyes on whole

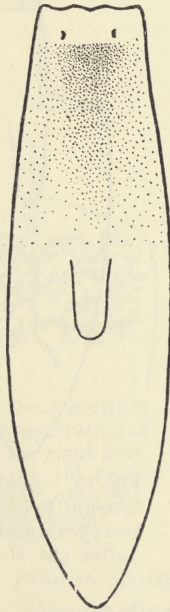


Fig. 9. *Dendrocoelum lacteum*. Head producing graded field.

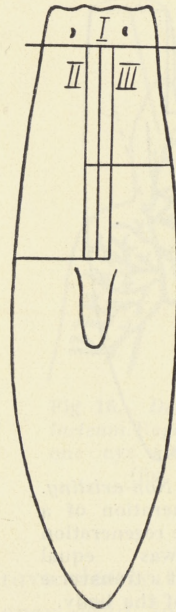


Fig. 10. *Dendrocoelum lacteum*. The head is removed. Three groups of specimens were cut as indicated by the three longitudinal lines, I in the middle line, II a little to the left of the median line, III a little to the right of the middle line. Thereafter a transverse cut removed half of the remaining forepart.

transverse sections originate close to the middle line, therefore in the same tissue as that contained in the 2,5 and 8-pieces. It will be seen that the 5-pieces start about 1 to 2 days later than the 2-pieces, and the 8-pieces again 1 to 2 days after the 5-pieces.

The 1-pieces behave of course in the same manner as the 3-pieces, because the body is bilaterally symmetrical also in respect to regeneration power (Br. 1942 b). They were therefore calculated together, and so also with the 4 + 6 and 7 + 9-pieces.

1 + 3 (broken lines) are cut at the same level as the 2-pieces, but nevertheless they start regeneration about one day later, and then only with one eye each; the second eye is regenerated after two more days (thin unbroken lines), and so the regeneration of symmetrical formations in the lateral 1 + 3-pieces is retarded 4–5 days.

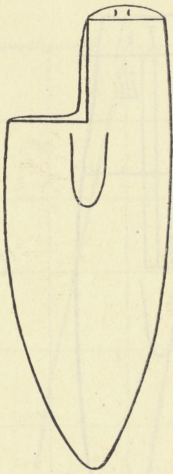


Fig. 11. *Non-existing*. The regeneration of a head if the regeneration power was equal throughout a transverse section of the body.

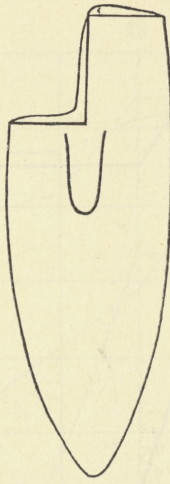


Fig. 12. *Dendrocoelum lacteum*. Regeneration of one eye close to middle line in the onset of regeneration; soon after another eye will appear just to the left of the first produced.

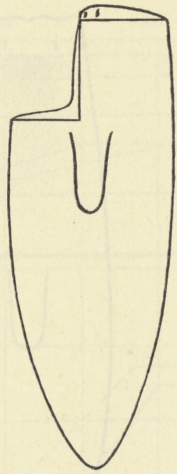


Fig. 13. *Dendrocoelum lacteum*. Regeneration of two eyes simultaneously after cut II (fig. 10).

Just the same is the case with the 4 + 6-pieces, they are retarded about 4 days in relation to the 5-pieces.

Still worse with the 7 + 9-pieces, they are 8 days behind the 8-pieces from the same level of the body.

It is noteworthy that in all instances the lateral pieces never attain the same absolute regeneration power as the corresponding middle-pieces.

I have tried to differentiate the experiments further by cutting the forepart of the body into 25 pieces, but without success; all the pieces disintegrated. This does not matter because there is not the slightest reason to suppose, that there should exist marked irregularities in the way of tapering of the regeneration field as it is revealed by the experiments in cutting the forepart into only 9 pieces. It must be borne in mind that this cutting in reality represents many variations in size of the pieces, because it is impossible to make the cuts exactly equidistant from animal to animal. In some specimens the lateral pieces comprise somewhat more than one third of the body-segment, in others a little less; in these latter the regeneration is much retarded.

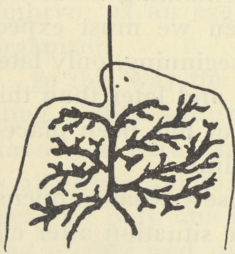


Fig. 14. *Dendrocoelum lacteum*. Regeneration of two eyes, the right first, after cut I (fig. 10). Fore-part of body with alimentary canal.

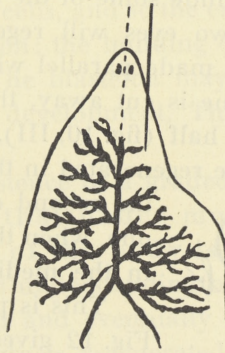


Fig. 15. *Dendrocoelum lacteum*. Regeneration of two eyes simultaneously after cut I (fig. 10). This specimen is left hand.

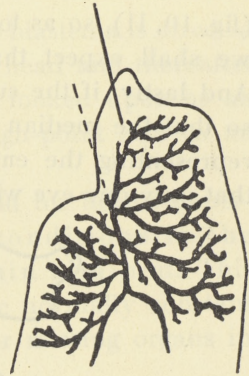


Fig. 16. *Dendrocoelum lacteum*. Regeneration of one eye after cut III (fig. 10).

The results from these experiments prove clearly that there exists a regeneration field, a head-frequency field in *Dendrocoelum*, which may be pictured as in fig. 9. It is very much like that made probable in *Bdellocephala* (fig. 5).

In order to test the theory of the graded regeneration field a few experiments were made.

15 *Dendrocoelum* were cut longitudinally after being decapitated. The cut was made either in or a little to the side of the middle line. With the aid of the alimentary canal, which can be easily seen through the unpigmented body, it is possible with fair exactness to determine the middle line. After removal of the lateral piece the animal was severed transversely by a cut midway between the anterior transverse cut and the gut (fig. 10).

If it were not known that the head-frequency also graded laterally one might expect that a blastema with two symmetrically placed eyes in the middle of the blastema should be regenerated as in fig. 11. With the knowledge of the graded field in mind we must, however, expect that the eyes regenerate near the middle line, close to the cut.

If the cut is made *in* the median line (fig. 10, I) we must expect that first one eye is regenerated, soon after another towards the median line. If the cut is made outside the median line

(fig. 10, II), so as to include some of the other side's tissue, then we shall expect that two eyes will regenerate simultaneously. And lastly, if the cut is made parallel with the median line but so that the median tissue is cut away, the remaining tissue not representing the entire half (fig. 10, III), then we must expect that only one eye will be regenerated in the beginning, only later the second eye, and later than this one, when the cut has been placed in the median line.

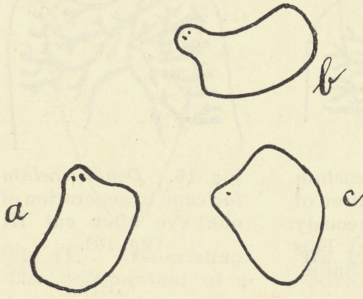


Fig. 17. *Dendrocoelum lacteum*. a "3-piece". b "4-piece". c "9-piece".
Explanation in text.

This is precisely what happens. Fig. 12 gives the situation after cut I schematically, and fig. 14 a camera lucida drawing after the second eye has appeared. Figs. 13 and 15 represent the situation after cut II, and fig. 16 after cut III.

Fig. 17 gives some instances of the eye regeneration in the small 3, 4 and 9-pieces (see fig. 6). It must be noted that the eyes always appear in the antero-median corner of the pieces, here the regeneration power is greatest. The median eye is smaller than the lateral because it is regenerated later. In fig. 17 c (a 9-piece) the second eye has not yet been regenerated.

IV.

We now come to the main thesis of this paper.

Let us imagine an accident: the animal is attacked and a part of the fore body with the head is snatched away by the enemy. The torn surface may be transverse, oblique, curved or straight; on the wound surface there will always be a place where the head-building ability acts most energetically and in shortest time. This place will henceforward be named the high-point.

Now it will be remembered from the experiments that here the regeneration starts earlier than elsewhere in the wound. That is to say: in the high-point the first organization of the regeneration cells takes place. From here organizing effects

spread to neighbouring cells, and so the entire blastema is directed into its future destination: the building of a head and thereafter what else is needed. The blastema may be looked upon as an embryo, as an egg in development. The high-point acts as an organizer.

In this way the existence of a graded field for head-regenerating ability secures that under most conditions in the nature a wholeness of the body is again attained. This is all important for the understanding of the normally constant fact that only one head and eventually other missing organs in due quality and quantity is regenerated.

It must be borne in mind that it has been shown that all parts of a cut surface are able to regenerate heads. When they normally do not do so, it is due to the existence of a graded regeneration field with its graded set of high-points and not to directing forces of some obscure superbodily principles.

In adopting this theory we have, I think, annihilated all more or less philosophical speculations as to the possible existence of a superbodily "Ganzheitsfaktor", entelechie and other unscientific speculations so richly set forth in the between-wars literature, also the more formal notions of "force-lines", "correlation-lines", and so on.

V.

It has been said that the regeneration phenomena in planarians were so prodigious, so manifold and contradictory that it were almost impossible to get information applicable to general regeneration. And surely, in plunging into the vast literature one may in fact become somewhat downhearted.

In this section I shall try to show that the most varied and complex experimental results met with in the literature can be reconciled in the light of the existence of the graded regeneration field in planarians.

T. H. MORGAN'S papers 1898—1902 must still be recognized as standard works in the literature on planarian regeneration, both as to the varied and ingenious experiments and as to the hypotheses set forth. They still give much sound information and

still stimulate the thoughts of the reader in these later times of notions and hypotheses then unknown to the author. Among the funds of experimental results only a few shall be brought forth here to illustrate our thesis.

Long narrow pieces often regenerate one or two heads on the side in such a way that the new longitudinal axis may be made oblique towards the old one. This is so because the high-point of the field lies near the former middle line of the animal.

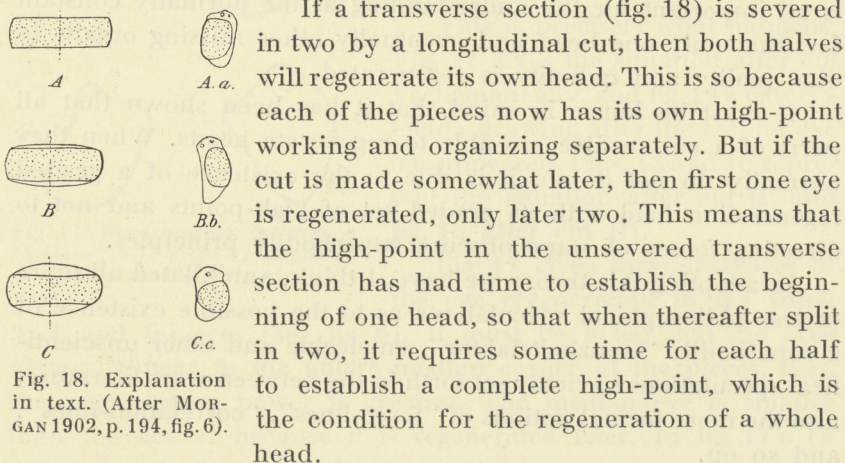


Fig. 18. Explanation in text. (After MORGAN 1902, p. 194, fig. 6).

MORGAN's examples of twin buildings after a cut through the midline of the body again demonstrate the existence of a graded field. The same is the case with some experiments, where MORGAN shows that when a longitudinal median cut is made antero-posterior and, say, half way down the decapitated body, then the two halves may each regenerate its own head, also when the one part is shortened.

A few quotations from MORGAN's paper 1902 give instances of facts then obscure, but now understandable in the light of our knowledge of the graded field.

P. 196: "If an oblique cross-piece (fig. 19) is cut into two half-pieces as indicated in Fig. 7 B, x—x, we should expect to find that in the righthand piece the head would appear on the outer right-hand side, but since there has been shown to be in half cross pieces some influence that brings the head towards the inner side we should expect to find the new head, adjusting itself to both tendencies, appearing about the middle of the

anterior edge. In the left-hand half-piece both of the former influences would tend to bring the new head towards the inner side. The figures show, Fig. 7 B a, a', b, b', etc. that the anticipated results follow, although owing to the change in shape of the old part it is not always clear just where the new head arises. In both cases a single eye first appears—*always on the outer side of the piece* (italics by Br.). In the right-hand pieces the head lies nearly in the middle of the anterior edge; while in the left-hand pieces it arises at the inner angle.”

P. 206: “If the anterior end is cut from the worm (fig. 20), the body partially split into two equal parts, and one of the halves is cut off near the bottom of the last cut, as indicated in fig. 14, there are exposed two cut-ends at different levels. The pieces will fuse

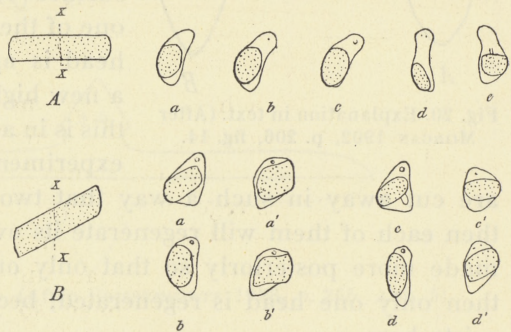


Fig. 19. Explanation in text. (After MORGAN 1902, p. 196, fig. 7).

along the middle line unless they are for a day or two repeatedly separated. If the pieces fuse along the middle line a single head develops, and this head is always at the anterior cross-cut. New tissue appears along the inner edge of the longer half-piece that is at its most posterior end continuous with the new tissue covering the posterior cross-cut. A new head is not produced by the latter, and this region is slowly changed into the side of the new worm. If, however, the longer and the shorter half-pieces are kept from fusing along the middle line a new head generally develops on the posterior cross-cut.”

An interesting paper of Y. LI 1928 shows that he has many thoughts about the directing forces in regeneration. He gives tables for correlations between the various parts of the planarian body and points out that when certain of the lines which may be drawn in certain ways through these correlations, then only regeneration occurs. These ideas are, however, purely formal and symbolic, interesting because they in a way forecast ideas about fields and gradients.

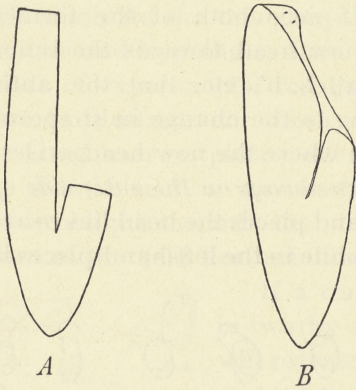


Fig. 20. Explanation in text. (After MORGAN 1902, p. 206, fig. 14.)

LI's experimental results all fit with the fact of the existence of a graded field. Most striking is perhaps this (fig. 21): The head is cut away, a new cut along the median line separates the forepart in two, each of these regenerates its own head, because each half anterior surface constitute a high-point. If one of these is cut away then a new head is again regenerated because a new high-point is exposed. So far this is in accordance with MORGAN's experiments. If now both foreparts

are cut away in such a way that two stumps are still existing then each of them will regenerate its own head. But if the cut is made more posteriorly so that only one surface is forthcoming then only one head is regenerated, because only one high-point exists here.

LI shows that several wounds do not retard the regeneration of a head. He does so with an ingenious experiment: he makes several cuts transversely through half the animal, the cuts glue together. Next day the cuts are prolonged over the left side. Then the head is cut away. A new head is regenerated just as early and well as in normal controls. This procedure is varied. LI

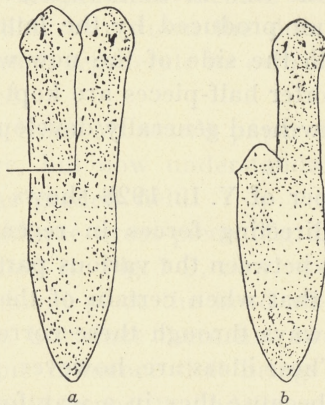


Fig. 21. Explanation in text. (After Y. LI 1928, p. 247, Abb. 11).

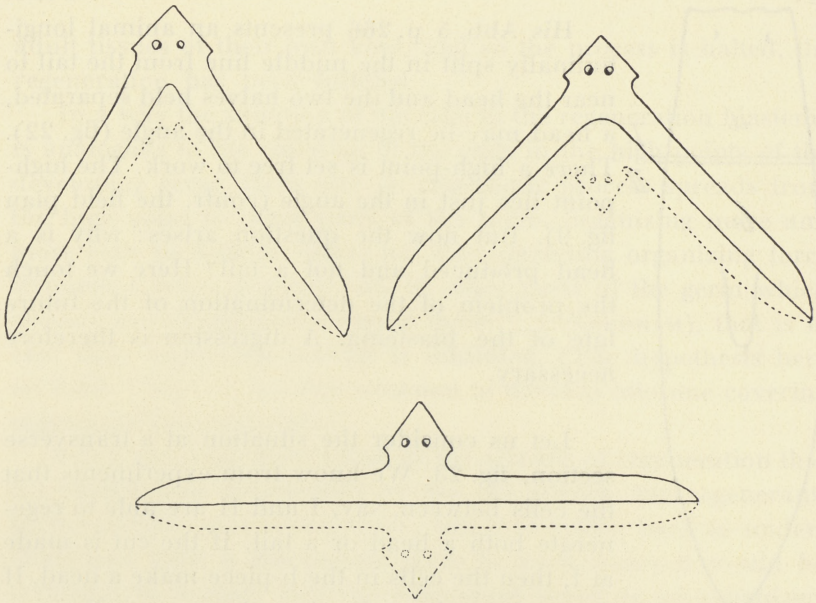


Fig. 22. Explanation in text. (After BEISSENHIRTZ 1928, p. 265, Abb. 5).

draws the conclusion in a formal way in saying that the coalescence is no hindrance for regenerative correlation. His results show that the graded field is a property laid firmly down in the body, and it is not disturbed by minor wounds healing throughout the body.

LI further describes several heteropolar buildings which do not cooperate in regenerative correlation, that is to say: by varied cutting more high-points are set free to work, and they do so independently when they are not in the position making one of them superior to the others. LI's results force him to draw parallels between the regeneration power and radiant forms of energy, again a formal conception.

In the same year, 1928, BEISSENHIRTZ published extensive series of experiments on regeneration in the species *Planaria gonocephala* and *Polycelis nigra*. If anything, these series show the formidable regeneration power of planarians. Without knowledge of a common principle these results are so confusing that it is well understandable that one may give up in despair. Let us consider some of BEISSENHIRTZ's findings in the light of the graded field-theory.



Fig. 23. *Dendrocoelum lacteum*.
Explanation in text.

His Abb. 5 p. 266 presents an animal longitudinally split in the middle line from the tail to near the head and the two halves held separated, a head may be regenerated in the angle (fig. 22). There a high-point is set free to work. The high-point lies just in the angle (confr. the field plan fig. 9). But now the question arises: why is a head produced and not a tail? Here we touch the problem of the determination of the future fate of the blastema. A digression is therefore necessary.

Let us consider the situation at a transverse section, fig. 23. We know from experiments that the cells between, say, I and II are able to regenerate both a head or a tail. If the cut is made at I, then the cells in the b-piece make a head. If the cut is made at II, then the same cells, now in the a-piece, produce a tail. How do these cells know if a head or a tail is to be made? I think the matter may be conceived thus: when the cut is made and two surfaces suddenly exposed, then the inherent regeneration mechanism goes into action at once along the entire surface. Soon the high-point takes the leading and in a very short time the high-point stands before the question what to regenerate. We know from experiments that in some species $\frac{1}{2}$ or lesser of the body is able to regenerate a whole animal, we must therefore conclude that the high-point has the power to organize a whole animal. We must therefore ask: why is only a head or a tail regenerated and not a whole animal? We may conceive that the high-point cells very soon after their first activity are inhibited in their ability (ev. their "desire") to organize the cells in the blastema to a whole animal, inhibited by those sorts of cells and organs already existing. We are here helped by experiments. In 1939 I have shown that adult tissue transplanted to a wound inhibits regeneration. Therefore a hind-part must inhibit the high-point in making a new hind-part, and therefore it makes a head, and vice versa. Then the new head (high-point) goes on determining the cells just to the level where they meet with

adult tissues of their own kind, and so the process is halted, the regeneration has been fulfilled.

In a way the situation of the cells in the regeneration blastema is analogous to the situation of the cells in the high-points of the developing germ. Here also an organizing process spreads from the high-point to other parts of the germ, organizing more and more cells to definite tasks; but this spreading organizing force terminates when it meets cells in other parts of the germ which already are determined ("closed doors", J. NEEDHAM), that is to say: their organizing activity is inhibited. (The hypothesis here set forth I hope at another occasion to develop into one covering regeneration in general).

It is a common conception in the science of regeneration that the blastema is determined by the old tissues, the regenerant. So it must be, in a broad sense. But it is rather hard to understand how such a mechanism were to work, since it would be necessary to assume that the regenerant acted as an organizer. It has in fact been said (f. i. HUXLEY & DE BEER) that the planarian head acts as an organizer, but I have shown in 1939 that this is not so.

If we cut the head and a portion of the forepart away, then the first thing to be regenerated is a head (BRØNDSTED 1942), only later the other missing parts are regenerated by a sort of intercalation. This is rather hard to understand if we assume an organizing force from the old tissues; one should expect that the new tissues which shall continue the old parts were organized first and thereafter those lying anteriorly thereto, and so on, the head being the latest to be regenerated. But we know from all such experiments that the first part to be produced is always the head. (I should here like to call attention to an attractive task of investigation: to elucidate the hierarchy of organs regenerated after certain losses). Only hereafter the head or high-point organizes the rest of the missing body.

It is timely here to draw attention to a curious fact: when two half animals are transplanted together, a transverse cut made through both parts, then on the wound surface only one head is regenerated, regardless of the fact that the regeneration cells come from two animals. This experimental result indicates that the regeneration cells are embryonic in character and collaborate

constituting only one high-point (BRØNDSTED 1942 a); they make therefore no rigid morphological framework of adult tissues. The situation is comparable with that where two eggs in the two-cell phase are held crosswise together and so fuse to form only one giant embryo.

It may now be asked: why is not a head being regenerated from every ever so little wound in the sides of the body in the head-field? (The planarian body is soft and very often exposed to such minor molestations). Because such small wounds only set free high-points of very little intensity; the regeneration cells in these lateral parts start so slowly and make so little progress in organization and determination that they do not get the necessary start in these processes before the inhibiting forces from the neighbouring tissues have had time enough to exercise their influence and so block the further organization of the blastema into other and higher organs than those which are missing. Therefore it is understandable that heads only occur by triangular cuts when these are deep enough, that is, when the cut reach such parts of the graded regeneration field where the intensity is high enough to overcome the inhibitory forces from the adult tissues. Such is also the case with the experiments carried out by me (1942 a) where a window is made in the fore-part of the body. Here a new head may be regenerated notwithstanding the fact, that the old head still is in place figs. 2 and 3.

Let us now return to BEISSENHIRTZ's experiments in question. The high-point in the angle (fig. 22) has initially the power to make a whole animal. Now, which inhibiting forces are most closely at hand? The high-point is in touch with both halves of the hind part, therefore inhibited in making that part of the body. It is, however, not in touch with the head, and therefore it regenerates a head. The situation closely resembles that of the head in the window related above.

Another of BEISSENHIRTZ's experimental results may be considered. A T-formed cut is made as indicated in fig. 24. Two high-points are established, each of them form a head and not a hind-part, the cause being the same as in the foregoing experiment. Two high-points are forthcoming because the transverse cut separates them from one another.

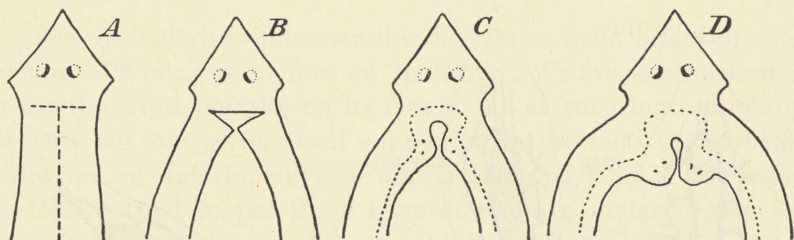


Fig. 24. Explanation in text. (After BEISSENHIRTZ 1928, p. 292, Abb. 24).

Also KAHL's paper from 1935 gives a wealth of experimental data. The work is, however, produced in so formal a style that it is often rather obscure what the real meaning of the author may be. The author uses very much triangular cuts into the side of the animal. In this way he produces the most singular chimaeras, in themselves a beautiful proof for the non-existence of a superbodily "Ganzheitsfaktor".

His experimental animal is *Planaria gonocephala*, which possesses a head-regeneration field stretching nearly to the hind part of the animal.

One example may serve to show how the existence of the graded field can explain the producing of a rather curious monster. A triangular cut is made in a decapitated animal, fig. 25. In the last figure we see the fully developed monster consisting of 1. an animal organized from the cut in the forepart, and 2. of a twinbuilding arising from two high-points, one from each side of the triangular cut. They have started separately, but later the two blastemas begin to fuse. The regulating forces are at work in the embryonic tissue of the blastemas.

SILBER & HAMBURGER 1939 also in *Euplanaria tigrina* produce the same monster buildings by triangular cuts as do BEISSENHIRTZ. Also these authors produce monsters with several heads when severing the high-points.

GOLDSMITH 1940 finds that wounds made by the lancet, electrocauter or warm needle are the starting points for regenerative buildings of longitudinal form which now and then are producing heads. All such buildings can be understood because the wounds set free embryonic, regenerative tissue from the field. It is by no means remarkable that various forms may ensue from such wounds, it all depends on the form, size and situation of the

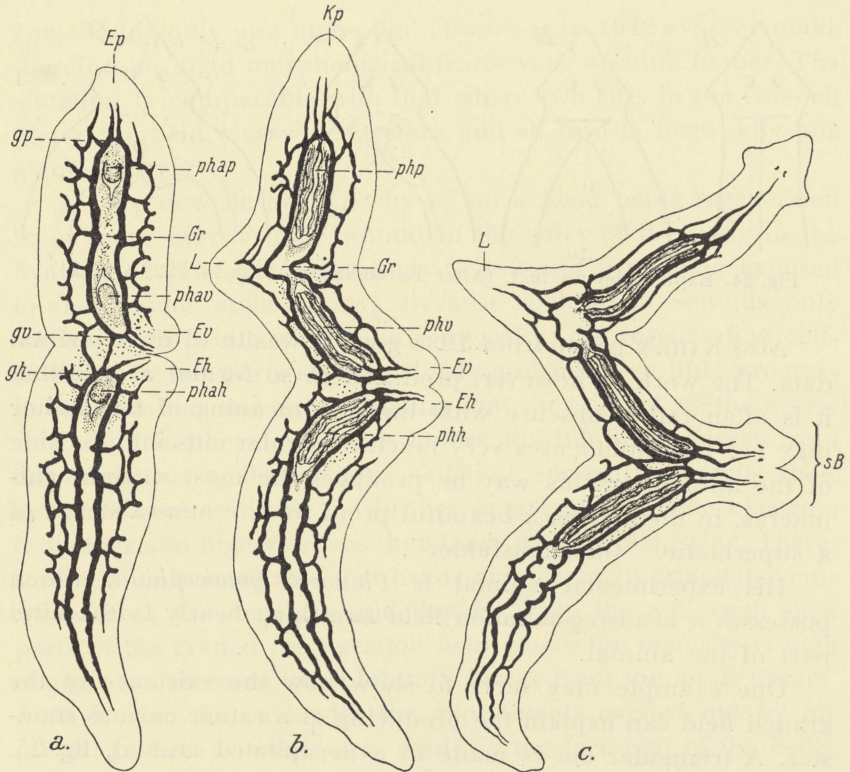


Fig. 25. Explanation in text. (After KAHL 1935, p. 655, Abb. 18).

wound in relation to the regeneration field and to the inhibiting forces in the adult tissues from which they emerge.

We must now proceed to consider certain curious monsters which are hard to explain on the basis of the graded field, and which hitherto have been an enigma; these monsters are the so called Janus Heads.

It is an old observation that a planarian head, narrowly cut off, may produce a head on its hind surface. So a chimera arises consisting of two opposite heads without a body. It is also seen now and then that a narrow transverse segment regenerates heads on both the anterior and the posterior surface. (Fig. 26).

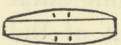
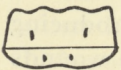


Fig. 26. *Dendrocoelum lacteum*. Janus head from head and from a narrow transverse section of forebody.

The difficulty in understanding the Janus head arises in connection with our dim notions on "polarity". We are so accustomed to use the word polarity on its face value as covering something undefinable, but giving itself expression in the facts that a head arises on an anterior surface and a hind part on a posterior.

I have tried to put these facts down to a material base in assuming an inhibiting force in the old tissues towards the "desire" and ability of the blastema cells to regenerate a whole animal. This force is of course chemical in nature.

When so the abilities of the blastema do not manifest themselves completely it is on account of the inhibiting forces; but in the Janus head this seems not to be the case, on the contrary, here is regenerated precisely that which already exists. How is this?

As a working hypothesis I set this forth: when the cut is made just behind the eyes, then it hits the part of the regeneration field which acts most energetically. It may be supposed that the regeneration cells start so violently and fast that they determine themselves before the prohibiting influence in the small amount of adult tissue has got time to brake them. When later this influence does reach them, the determination is made, "the door is closed". It must be remembered, that the Janus head is a very rare phenomenon, in most cases the head has got time to brake the blastema, and so a hind-body is produced.

If we think about these processes as chemical influences from cell to cell, as we certainly must, this hypothesis does not seem far fetched, because the inhibiting influence, being chemical, certainly must require time before it can exercise its influence on the regeneration cells.

When the Janus head II is made the case is similar although not identical. It may first be stated that Janus heads so far only are known to occur in very anterior transverse pieces and only in very narrow ones. This being so it is understandable that a head may be regenerated both on the anterior and the posterior surface of the segment. When the segment is very narrow then only a very slight fall in the head-building ability is at hand from the anterior to the posterior surface. The two high-points therefore start almost simultaneously, and they start their work very energetically and immediately after the cut is made. The

situation may therefore now and then occur that the time difference between the start of the regeneration cells at the anterior surface and that of the cells on the posterior is too short to give preference in head-making to the blastema on the anterior surface as against that on the posterior, or to give inhibiting forces in the small amount of tissue in the segment itself time enough to exercise their influence on the posterior blastema.

If this hypothesis covers the facts then the Janus head is a further proof for the idea that the regeneration blastema in planarians starts with the "intention" and power to regenerate a whole animal, the regeneration of which is beginning with the building of a head.

VI.

The following questions now arise: 1. How may the regeneration-field be organized? 2. When does it arise in ontogenesis? 3. Is it connected with some metabolic field?

Let us briefly consider 3. A vast literature on the subject has been inaugurated by CHILD. Several of his pupils and collaborators have tried to squeeze several incompatible experimental facts into the common postulate that a metabolic gradient of coarser nature is in some way responsible for the head-frequency curve. The postulate has spread unto nearly all fields of morphogenetic investigations and have met with enthusiasm and the opposite. The reader may be referred to J. NEEDHAMS great book (1942) concerning the broader aspects of the problem. Here I only wish to emphasize that a priori it seems very unlikely that such a metabolic gradient field should exist in the adult planarian body corresponding to the regeneration field in *Bdellocephala* and *Dendrocoelum*, where the head-building ability is extinguished before the gut is reached. Furthermore I have shown (1936) that investigations with methylene blue as to the reducing power of various parts of the body of *Dendrocoelum lacteum* do not in any way correspond to the head-building regeneration field. Personally I find it nearly certain that the regeneration field in some way must be anchored in some metabolic field, but of so subtle a kind that it must not be confounded with the coarser "dayly-work" metabolism, it can therefore not be mea-

sured by such means as susceptibility to various poisons and drugs, inhibiting chemicals or oxidation activities in the body.

The second question, when does the field arise in ontogenesis, can not yet be answered with certainty. But I have shown (1942 a) that in *Bdellocephala* the regeneration field is fully developed already in the worms just escaped from the cocoons. I therefore think it most probable that this field is an inherited property or function just as well as all other species-specific properties; I therefore think that it is laid down firmly in the constitution of the worm already during the embryonic development, just as well as the regeneration power itself.

How may the regeneration-field be organized? This question cannot be answered as long as we do not know anything about the basic problem how it may be that some animals have cells in their body able to regenerate, and others which are not. We do not make any progress by telling the common tale that the higher the organization the lower the power of regeneration. So much the less as this tale does not cover the facts. Is there f. i. any reason in saying that *Bdellocephala* and *Dendrocoelum*, where the ability to regenerate head is extinguished just in front of the gut, is higher organized than f. i. *Planaria gonocephala* or *lugubris* where this ability stretches throughout the entire body?

It is of no use at the present state of our knowledge to speculate much further about this problem, only it may be assumed that the ability to regenerate is likely to be a reminiscence from the embryonic state of the cells and body, therefore in a way a projection into the adult of the regulating powers of the early germ.

The fact that a gradient in regeneration ability exists throughout the body has been ascribed to a postulated existence of a graded number of regeneration cells, and it has been assumed that there exists two sorts of regeneration cells in planarians, "head-cells" and "tail-cells". I think that more investigations are necessary to elucidate these problems.

VII.

The knowledge about the planarian head-frequency curve has been bound up with the various conceptions of morphogenetic fields, most markedly perhaps by HUXLEY & DE BEER 1934.

What is a morphogenetic field?

An attractive discussion with literature is given by J. NEEDHAM 1942, p. 127, the net result of which is that no clear cut definition acceptable for all can be given. The reason for this unsatisfactory state of affairs is of course that the conception of a morphogenetic field is based on observations of diverse phenomena, the underlying causes of which we do not know beyond the meagre one that we have a conviction that they must be of chemical nature.

As long as our positive knowledge is so meagre, it is, I think, wise not to put our definition of morphogenetic fields into too narrow and rigid frames. Our conceptions must be flexible and adaptive to some degree to meet the demands of the varying experimental results obtained.

When ventral epidermis in an amphibian early embryo is transplanted over the optic cup and then transforms into a lens, it is said to come into the "field" of the optic cup. When a piece of the dorsal lip in a vertebrate embryo is transplanted under the ventral epidermis then this latter transforms into a neural plate etc. The dorsal lip is said to constitute a morphogenetic field. When a young regeneration blastema of a hind leg from a salamander embryo is transplanted to the wound of the anterior limb, then the blastema will be transformed into a fore-leg; the blastema is said to have been transformed under the influence of the fore-limb's field.

Deducing from such transplantation experiments it is assumed that the various morphogenetic fields also exercise their power in much the same way in their ordinary place and on the ordinary normal material.

A morphogenetic field, then, is a certain force which under embryonic or regenerative circumstances expresses itself as a power able to make the cells of a certain district of the body mould themselves (or other cells ev. coming into contact with this district, and able to respond) into definite organ or organs.

GURWITCH's original conception is, I think, mingled up with some obscure ideas in somewhat remote accordance with power-fields of the ordinary physical nature, and perhaps in some way mentally connected with his ideas of "mitogenetic rays". The elaborate discussion by WEISS (1926) reveals that WEISS himself

must in some degree bear in mind more or less vague feelings in the same direction.

To my mind the soundest conception is laid down by HUXLEY & DE BEER (1934 p. 276): "... the simplest assumption is that there exists a graded concentration of the specific chemical substances responsible for limb-production and laid down by chemo-differentiation".

HUXLEY & DE BEER mostly use the word gradient-field (p. 274) because "various processes concerned with morphogenesis appear to be quantitatively graded, so that the most suitable name for them is field-gradient systems, or simple gradient-fields".

To secure the field-conception as a sound working-hypothesis I think it wise to emphasize that the field exists only linked to living cells; it is in fact part of the total sum of processes in which living cells are able to express themselves. If we do not rigidly bear this in mind when speaking of and dealing with morphogenetic fields, then we are in danger to handle a conception which is too apt to evade realities.

What then are the realities in the "field"? To my mind this: a chemical transmission by contact from cell to cell, starting from a single cell or a group of cells, spreading to all sides as long as other cells are able to receive the chemical stimulus and react thereupon. The reaction of a cell in morphogenetic respect is twofold. 1. The cell is differentiated and determined, α : the stimulus makes the cell transform into another function and perhaps another shape, so that it from now on has a specific work to do. 2. It gives off the stimulus in an altered form, transformed, to new cells, so making these in their turn differentiate.

It is reserved for future research to elucidate the chemical processes involved here. NEEDHAM gives some possibilities.

I may here lay stress on the point that the chemical stimulus from cell to cell in the morphogenetic process must vary from one cell to another, or at least from a small group of cells to the next small group, else it is impossible to understand the multitude of cell-sorts arising f. i. during the development of the brain. Bearing in mind the endless variation possibilities of the protein molecule it is not inconceivable to postulate an evershifting chain of chemical stimuli from cell to cell. The great riddle is how

the shifting is brought about in so rigid an order that a constant result, the well formed and organized organism, is nearly always gained. We can only say that the shift is governed by the genes.

The most amazing feature about the chain reactions from cell to cell is perhaps that they are so precisely timed. 1. The reactive state of the cells—their competence (WADDINGTON)—is generally limited in time. 2. Given a certain undifferentiated cell ready to react on various stimuli; it may after some time be reached by a certain chain reaction coming from a certain field, it reacts accordingly. Then it may be reached by stimuli from another field, but now it does not react because its destination is fixed by the first stimulus, the door is closed (J. NEEDHAM). If now the various transmission chains were not accurately timed great disorder in morphogenesis would happen. Such disorder in timing may arouse when the development takes place at extremely different temperature, by application of drugs or other chemicals. This situation is by RUNNSTRÖM called “Gefälleanarchie”; an example is given by me (1943).

Now, how is this picture of the morphogenetic field to be applied to the regeneration processes in planarians?

As before said, the phenomenon called head-frequency has been mentally connected with the field-gradient conception in ontogeny. HUXLEY & DE BEER generalize in this way: “In general, the term field implies a region throughout which some agency is at work in a co-ordinated way, resulting in the establishment of an equilibrium within the area of the field. A quantitative alteration in the intensity of operation of the agency in any part of the field will alter the equilibrium as a whole. A field is thus a unitary system, which can be altered or deformed as a whole; it is not a mosaic in which single portions can be removed or substituted by others without exerting any effect on the rest of the system”. And further: “In all examples so far studied, the agencies in question appear to be graded quantitatively in somewhat simple patterns, frequently (Hydroids, Planarians, many eggs) in the form of a single gradient coinciding with the long axis of the organism.”

Now we must be careful and define what a gradient-field means in embryology and what it means in regeneration, especially in the planarians.

Embryonic gradient-field: In amphibian eggs f. i., the gradient-field means the forces acting throughout the egg from a high-point at the dorsal lip; in echinoderm eggs there seems to be two gradient-fields the one with the high-point at the vegetative pole, the other at the animal. These embryonic fields are in steady progress, they are actually at work, they act upon cells undergoing differentiation, they are realities to be studied such as they are.

Regeneration gradient-fields in the adult body: These are static; they constitute patterns of abilities, which only disclose themselves, when a cut is made and regeneration starts.

The regeneration gradient-field is therefore quite another thing as the graded forces operating in the embryonic field. But when regeneration starts and the cells begin to work in the blastema, then here a gradient field is set up in this by these cells quite analogous to the cells in the embryonic gradient field; the blastema is analogous to an embryo.

It is therefore necessary to hold these two conceptions separated in our mind: the embryonic field, and the static, potential regeneration field. In the embryonic field the forces grade qualitatively; in the static regeneration field they grade quantitatively.

It is clear, and must be emphasized, that the phenomenon known in the planarians as the head-frequency curve, and now by me found to be a static, potential regeneration field, do not correspond to a graded embryonic field.

VIII. Conclusion and Summary.

In the head-frequency phenomenon discovered by CHILD we meet a graded ability down the antero-posterior axis of the body to regenerate a head. This ability lies firmly anchored in the adult body. It has now been shown in this paper, that the ability is graded not only along the antero-posterior axis of the animal, but also laterally. The field is static and potential and has the form in *Bdellocephala* and *Dendrocoelum* depicted in figs. 5 and 9.

The knowledge of this field, its strength and extension, is a necessary condition for the understanding of the most varied regeneration phenomena in the planarians.

It is shown that wherever a cut is made in the forepart of the body of the two mentioned species, a high-point for regenerative force is revealed; here in the high-point the regeneration starts, and the processes in the high-point are started so much earlier than those in other places along the cut that they overcome other tendencies to regenerate heads, and so it is secured that only one head is regenerated: the "wholeness" is restored.

By the discovery of this phenomenon it is shown that we no longer need to have recourse to dim notions as "entelechie", "superbodily" influences, "wholeness-factors" and so on, when we seek to explain why regeneration normally leads to a satisfactory result viz., the restoring of the animal in normal form and function.

The meaning of embryonic fields and static, potential regeneration fields are discussed and the difference made clear.

It is outside the scope of this paper to see, if the conception of the graded static regeneration field may be applied to regeneration phenomena in other organisms. Here lies a broad field for future research, able to give us deep-going insight into the riddles of regeneration.

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