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FURTHER EXPERIMENTS ON REGENERATION-PROBLEMS IN PLANARIANS

BY

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The scope of the experiments described in this paper is to investigate some basic problems in regeneration, especially the problems of regulation and inhibition in adult tissue and that of "organisatorstoffe" in regeneration.

The problems had to be attacked by a much varied set of cuts and transplantations, which may perhaps at first seem not to have much in common.

To understand fully the bearing of the experiments it is perhaps necessary to recall the phenomenon called by CHILD the head-frequency of planarians: when a planarian has had its head with a shorter or longer part of the body cut away transversely then it may regenerate a head from the wounded surface. The ability to do so may be different at various levels of the body, and so a "head frequency curve" may be plotted.

The head-frequency of e. g. Bdellocephala starts with 100 per cent anteriorly, decreasing a little caudad, then suddenly dropping to zero just before the pharynx.

I.

In a previous paper (1939) I have shown that when a transverse cut is made on a planarian, then the regeneration from the wounded surface may be stopped by the grafting upon this surface of a piece of adult tissue with reversed polarity.

This was observed in several cases under different conditions. In one instance, however, there was some doubt: when a head was grafted upon the anterior surface of a transversely cut body-segment of *Planaria lugubris*, but in such a way that the ventral side of the head pointed upward, that is with reversed dorso-ventral axis against the body, then it happened in two instances out of 15 experiments that the body regenerated a head beneath the grafted head. It was natural to suspect that in that case the cut surface of the grafted head did not completely cover the cut surface of the body, so that this latter had some small free surface, from which the regeneration of a head had started.

In order to test a certain question to be mentioned later it was necessary to clear up this problem.

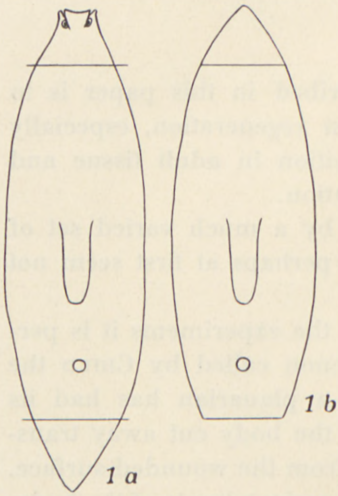

Fig. 1. *Bdellocephala. a,* the animal with transverse lines indicating cuts. *b,* tail transplanted to the anterior part of the body from which the head has been removed.

The animal used was *Bdellocephala punctata* (Pall.), the body of which is 2—3 cm in length. The head-frequency curve of this animal is described in my paper from 1939. Partly on account of the peculiar form of this curve and partly also on account of the hardiness of this species, I have made extensive use of this animal which may be taken from late autumn to the spring in quantity and in sufficiently large specimens.

The experiment was done as indicated in fig. 1. The head with some of the forepart was removed by a transverse cut. The tail was likewise removed by a transverse cut and grafted upon the anterior cut surface. Care was taken that the two cuts were as nearly as possible of the same size, so that no considerable part of the wounds remained uncovered when grafting had taken place. The technique employed was that described in my paper from 1939.

After 8 days only 4 specimens were in a proper state, the others were either dead or the tails separated from the main pieces. 38 days later there appeared some unpigmented tissue between tail and main piece in all four specimens, but no trace of head or eyes could be detected. 48 days after the starting of the experiment the situation was the same.

From this experiment it can be concluded that the forepart of Bdellocephala is not able to regenerate a head when the cut surface is completely blocked by adult tissue.

The controls, decapitated animals without grafted tails, regenerated heads 6—8 days after the operation.

After the settling of this question another problem could be attacked. The problem is this: will a head, grafted upon the side of Bdellocephala, reorganize itself and the body unto which it has been grafted, in such a way that it will become the "working" head of the animal?

The question is of considerable interest in so far as it may be able to throw light upon the problem of regulative powers in the adult planarian body.

From the foregoing experiment we know that a tail grafted upon an anterior cut surface inhibits the regeneration of a new head from this surface. Therefore I made the experiment as indicated in fig. 2.

The graftings were all autoplastic. Transverse cuts were made just as in the foregoing experiments, but in addition an oblique cut on the right side of the body was made to receive the head. The cut surfaces were as nearly as possible of the same size.

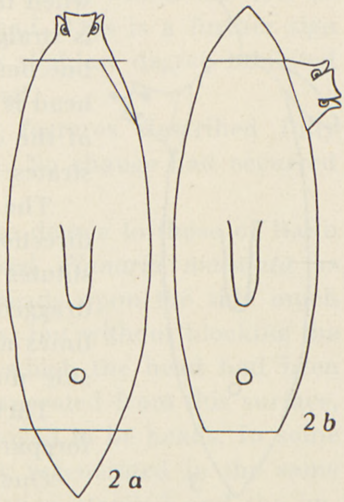

Fig. 2. *Bdellocephala*. *a*, the animal with lines indicating cuts. *b*, grafting of the tail upon the anterior surface, head upon the side surface.

The idea is that the tail blocks the regeneration of a head in the main axis of the body, and so the opportunity should be given to the sideward grafted head to take the leading so to speak and regulate itself and the body into a new functional entity.

The experiment is a rather difficult one, it could be foreseen that only very few specimens would succeed. 40 animals were operated on, but only two survived in the proper state. But some others showed interesting features in the course of the experimental period.

The two complete chimeras (fig. 3) show the same phenomenon. Tail and head have grown smoothly together with the animal; some unpigmented blasteme tissue has appeared between animal-tail and animal-head. It is plain that the head has not taken up a position as head for the animal. This fact can be seen both from the oblique position which it still holds and from the movements of the chimeras. When the worm itself is lying still, it may be seen that the head tries some forward movements of its own, it stretches forward and becomes alternately long and thin and short and thick. But when the whole chimera moves, then the path is straight forward when the head is inactive, but bending somewhat to the right when the head is also moving forward. The movements of the chimera in this case strikingly demonstrates the parallelogram of forces.

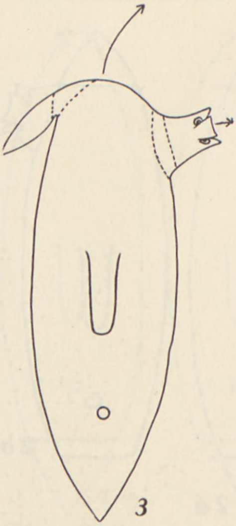

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Fig. 3. *Bdellocephala.* The chimera from fig. 2 *b* after 20 days. The short arrow indicates the movement of the head, the longer arrow that of the entire worm. The dotted lines mark the boundary of the unpigmented tissues.

The tail has no active influence upon the direction of the worm's movements; it constitutes only an inconvenient burden to be dragged along. The position of the tail is sometimes along the side, sometimes at the ventral side under the worm.

There is no trace of head or eyes in the forepart of the worm itself.

One of the other chimeras has lost its grafted tail. It has regenerated a head of its own which is well developed in spite of the grafted head. The forward movement of this chimera is not straight but bending in a curve somewhat in the direction of the grafted head. It is an interesting feature that this curve has a bigger radius than that of the just described chimera without its own head. This proves that the moving energy of the chimera with its own regenerated head is stronger than that of the chimera without its own head.

Three chimeras have lost the grafted head but retained the grafted tail. These are therefore in the same state as those issuing from the experiment indicated in fig. 1. Here the movement is straight forward along the main axis of the worm itself.

From these experiments may be deduced 1. that a tail grafted upon an anterior cut surface inhibits the formation of a head and 2. that a head grafted anteriorly upon the side of the worm remains a foreign element to the worm, and 3. that a grafted head does not inhibit the formation of a new head upon the free anterior cut surface.

Several animals have lost both the grafted tail and the head. They served as controls. They of course regenerated new heads. But it should be noted that these heads are not better developed than the heads regenerated upon the worm which has lost its grafted tail but retained its grafted head. This is a further sign that the grafted head has not in the slightest degree inhibited the regeneration of a new proper head.

All the experiments showed the features described fully developed 20 days after the operation. No change had occurred after 41 days.

These experiments contrast in some degree to those of Rand & Brown (1926). These authors used *Planaria maculata* as experimental animals. They grafted heads upon the side much as in the above described experiments, but without blocking the anterior surface of the animal from which the head had been cut away. Accordingly new heads regenerated from this surface, but were cut away as soon as they proved to be heads. In some instances new heads were continually regenerated in the same place, but in other cases the regeneration stopped and the implanted head swung into the main axis of the animal and seems to be the working head of the animal. It is not stated whether this phenomenon is an outward, apparent one, or whether it comprises a remoulding of the inner organs. The figures given are not decisive.

II.

A question of some theoretical interest is this: does the head of planarians exercize a reorganizing, morphallactic influence upon already existing structures in the body? To test this question two sets of experiments were made.

The material was again Bdellocephala. The animals were taken ultimo August, so the animals were not fully mature. Bdellocephala usually lays its cocoons in February—April.

In the first set of experiments the following procedure was employed: by transverse cuts a shorter or longer forepart of the body was separated from the rest of the animal. 20 animals were operated on as indicated in fig. 4*bI*, 20 as in *bII*, 20 as in *bIII* and 20 as in *bIV*.

It was tried to make exact measurements of the distance between cut and pharynx. This could not be done, however, neither with nor without anesthesia. In the first case the animals

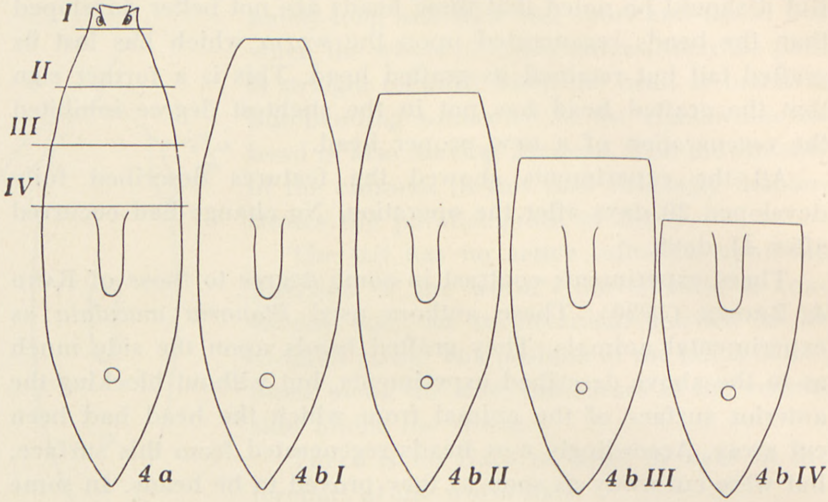

Fig. 4. *Bdellocephala*. *a*, diagram showing the four sets of cuts producing the four groups of experiments shown in *b*.

would contract somewhat irregularly so that the exact place of the cut in relation to the pharynx could not be determined, and in the second case the worms would move in being operated on. The cuts were therefore made by eye measure. The rather large number of experiments, 80 in all, should in some degree serve to eliminate the deviations.

The head-frequency curve of Bdellocephala terminates just before the pharynx. It could therefore be expected that group IV would not regenerate heads, and so was the case.

After 9 days 19 specimens of both group *I* and *II* had regenerated heads but only three of group *III*.

Now it may be asked: 1. does the regenerated head exercise some morphallactic influence upon the rest of the body in so far that this is remoulded in relation to the head, or 2. must the regenerated tissues adapt themselves to the old tissues? If the latter be the case then the distance between the regenerated head and the borderline of the old tissue, which is easily recognizable by its pigmentation, must be maximal in group *III*, minimal in group *I* when the regeneration processes have been completed.

The experiment proves that the question must be answered as foreseen under 2. 35 days after the beginning of the experiment the regeneration has been brought to a standstill. It is now seen, as shown in fig. 5, that the head in group *I* is separated from the old pigmented tissues by an unpigmented part of about the same length as the head. In group *II* this unpigmented part is about double the length of the head, and in group *III* it is about three times the length of the head. One feature was especially studied: the distance between the pharynx and the head. This distance is of course shortest in group *III* during the first part of the experimental period, but at the end of the experiments the distance has been regulated up, so that it is the same in all groups, comparatively of course, because the absolute distance in group *III* is smaller on account of the smaller size of these animals, which have lost more material by the operation than the other groups, and therefore have to bring up more material from the body to establish regeneration of new tissues.

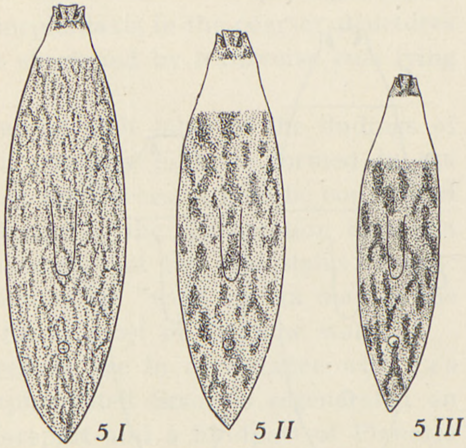

Fig. 5. *Bdellocephala*. Result of the experiment outlined in fig. 4 after 40 days. The unpigmented areas are the regeneration tissues intercalated between the now pigmented heads and the body.

From these experiments may be concluded that the regenerated tissues must adapt themselves to the already existing old tissues so that a harmoniously built animal comes forth not by morphallaxis in the old tissues but by a suitable moulding of the regenerated tissues, the cells of which must of course be derived from the old body. So in Bdellocephala a head in regeneration does not exert a reorganizing influence upon the old parts of the body.

A second set of experiments were made to test the question if an already adult head may possible reorganize the old body.

In order to secure conditions as close as possible to those of the first set of experiments the following procedure was employed.

20 Bdellocephala were cut as indicated in fig. 6*a*. Then the

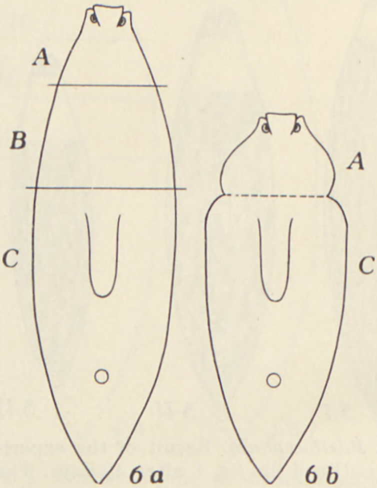

Fig. 6. *Bdellocephala*. In *a* the transverse lines indicate the operation. *A* the head to be grafted. *B* the body-segment to be removed. *C* the body to receive the grafted head. *b* the completed grafting.

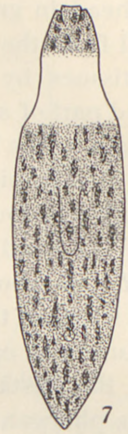

Fig. 7. *Bdellocephala*. The result after 44 days of the experiment outlined in fig. 6. Unpigmented regeneration tissue is intercalated between head and body.

head *A* was grafted to the anterior surface of *C* as indicated in fig. 6b. The piece *B* was not used in this experiment. By this procedure the animal was artificially shortened.

We may now ask: 1. will the head reorganize the whole body so that the organs are remoulded in accordance with the now shortened body length, or 2. will regenerated tissue be intercalated between the body and head to restore the normal animal without any remoulding of the coarser structure of the old tissues?

The result of the experiment shows that 2. must be answered in the affirmative. 6 days after the operation 9 successfully carried out chimeras were living. 14 days after only 5, two having been fixed in Zenker's fluid. Of these five one separated its head. The remaining four showed varied constructions in the now intercalated unpigmented regeneration tissue as shown in fig. 7. 44 days after the four animals were still living and they all show that the head distance had been regulated in accordance with the unmolested animal.

So these experiments show that neither heads in regeneration nor normal heads produce morphallaxis in the coarser structures of the old body when this is shortened by transverse cuts lying before the pharynx.

These results seem to contrast with some of the findings of T. H. Morgan 1898, where a pharynx may be formed in the old tissues, e. g. in regenerating side-pieces. It is to be considered if such morphallaxis is not due to the regeneration blastema penetrating into the old tissues so that the real status is this: most of the cells of the old tissues are "used up" in making the blastema which in its turn forms most of the new worm.

On the other hand my results are in accordance with Li's findings (1928) that new unpigmented tissue is regenerated on the grafting place where a forepart and a hindpart of Planaria lugubris are grafted together after the middle portion with the pharynx had been removed.

III.

A question of considerable theoretical importance is this: if two median halves from two separate animals are grafted together, and then the heads cut away, how will it be with the regeneration from the cut surface? 1. Will each half animal regenerate its own head, or 2. will both together form one single common head?

If the first should prove to be the case then it would be as if each of the two animals said to its own blastema: make a head for me. And so it is, when only half an animal regenerates singlehanded. Therefore one should expect to see two heads regenerated from the chimera.

It is of course known that when half an animal regenerates, then a new complete head will be formed. It is now also known from the foregoing experiments that a transplanted head or tail or whatsoever other part of the body will behave as a foreign part of the animal upon which the graft is implanted. It is therefore understandable if ones first thought is that the suggested experiment should result in two heads being regenerated, one for each half animal.

The experiment was carried out in two ways. In each set of experiments light and dark coloured animals were used, so that

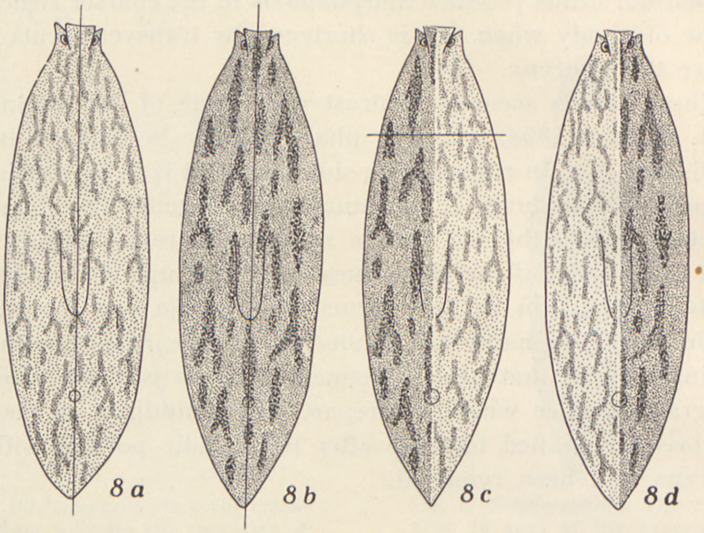

Fig. 8. *Bdellocephala.* A light coloured animal, *a*, and a dark coloured, *b*, cut in longitudinal halves as indicated by the lines. *c* and *d* after the completed grafting.

when grafted together the two halves could at every stage be distinguished from one another. In the first set of experiments 10 worms, 5 dark and 5 light ones, were cut in two by a longitudinal median cut. The light halves were grafted together with dark ones, fig. 8.

The experiment is exceedingly difficult, because the animals, although in deep narcosis, are bending semilunarly or circularly towards the wounded side. Several readjustments during the next 36 hours were needed. Only two well-shaped chimeras survived.

The next step in the operation is to cut away the heads by a transverse cut.

Ten days after the chimeras have regenerated a single head, fig. 9.

The other set of experiments were made in this way: 10

animals, 5 light- and 5 dark-coloured, were operated upon and grafted together as shown in fig. 10*a*. Here also the mortality was considerable, so that only two chimeras survived.

After secure coalescence had taken place the animals were operated on as shown in fig. 10*a*.

This experiment was made in order to see if the amount of tissue, which is very different in the dark and light part of the chimera, should make any difference in the result. Fig. 10*b* shows that the result was the same as in the foregoing experiment: one single common head was regenerated covering the cut surface without the slightest tendency for assymmetry towards the bigger part of the chimera.

Fig. 9. *Bdellocephala*. The chimera 8c has had its heads cut away by a transverse cut. From the surface a common head is regenerated.

These results are rather startling. They prove that both parts of the chimera take part in the formation of the single common head. They further prove that the two parts, the dark and the light coloured animal, are not able to direct their regeneration blastema as after their own ways. They show that such coworking blastemas have had the order from each worm: form a head. But they disobey an order formulated thus: form a head for me alone. It seems that the blastemas have the general order to form head, but they now, in fusing, collaborate and form one head.

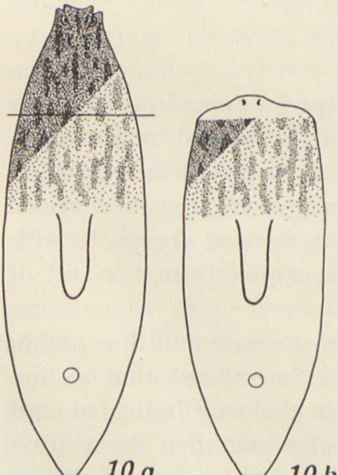

Fig. 10. *Bdellocephala*. A chimera was produced as indicated in *a*. The transverse line indicates the cut made. *b*, a common median and symmetrical head is regenerated.

What is going on? I think the most likely answer is this: the cells in the blastema are so to speak embryonic in their behaviour, they are loosened from the rigid order of the adult body, they have lost their original individuality although they have been born from two separate animals. They have been stamped only to form a head, and they do it regardless of the two separate sources from which they come.

The situation is, it seems, the same, mutatis mutandis, as when two amphibian eggs in the two-cell stage are placed crosswise together, so that the grey crescent regions touch one another. In this case the two embryonic groups collaborate and form one single giant embryo (MANGOLD and SEIDEL 1927).

I therefore think that these experiments are very strongly in favor of the view that the regeneration blastema is of embryonic character.

A further significant parallel may be drawn between this behaviour of the blastema and that of the developing egg. We know from the works of several investigators that each of the two first blastomeres of the amphibian egg has the power to develop into a whole animal, when separated, provided that the first cleavage furrow coincides tolerably with the future median plane of the animal. When these two cells collaborate in the normal egg to form only one animal, it must depend upon the ability of each blastomere to inhibit the total development of the other. So also in the blastemas of the just described chimeras. Each of them may, when separated, form a head, but brought together, they inhibit the tendency for totality in each other.

IV.

A well known feature in regeneration-phenomena in planarians is the fact that by splitting the foreend of the animal by a median cut the two halves may regenerate a new complete head provided that the split parts are prevented from growing together again. LUS (1924) has succeeded in producing several foreparts with heads stretching forward much as a boucquet from the rest of the body.

We here have to grapple with that most vague and dim notion in regeneration called inhibition. The fact about that notion remains that when the animal is split in the way indicated, but the two halves not prevented from coalescing, then the wound is simply closed and heals up, and the animal goes on with its ordinary single head. But when the two parts regenerate separately two normal heads are formed. This proves that every half and fourth and so on has the power to build head. Why do they not do so when separated by a cut but again allowed to coalesce?

We here use the word inhibition, we say that each half inhibits the other from exercising its power to make a head for itself. This phenomenon has of course the same underlying cause as when the two first blastomeres only form half an embryo each, when they are working together, although each of them has the power to form a whole embryo.

In both cases, that of the egg and that of the planarian, it is obvious that contact between the cells releases the mechanism of inhibition, whereas when they are separated in space this mechanism cannot unfold itself. It is therefore probable that the mechanism must work by contact from cell to cell and not by substances liberated and circulating in the body.

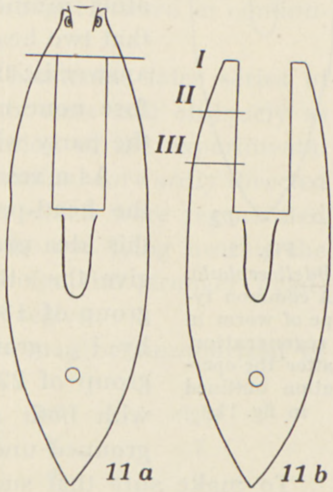

Fig. 11. *Bdellocephala.* After the operation indicated in *a*, *b* was produced. 3 groups were cut as indicated by the roman figures.

Meanwhile several facts both in embryonic development and in regeneration suggest that somehow inhibitory forces reveal themselves over certain distances without immediate contact between the areas which seem to inhibit one another.

In planarians for instance Rand & Ellis (1926) in a very suggestive study claim that the length relation of the two parts originated by splitting the animal decides whether the shorter part is able to regenerate a head or not.

In order to penetrate a little into this mystery some series of experiments were devised. The idea in the first series was this (fig. 11): Bdellocephala was operated on so that first the head was cut away and thereafter a median section of the body reaching nearly to the pharynx. So we get decapitated animals with two "arms". Bdellocephala is known not to regenerate head from a body-level just before the pharynx, the two "arms" may therefore be regarded as independent head-forming areas.

If hypothetical inhibiting substances should exist circulating independent of cells throughout the body, then it should be expected that substances from one arm should inhibit regener-

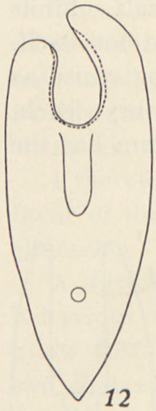

Fig. 12. *Bdellocephala.* A common type of worm in regeneration after the operation outlined in fig. 11.

ation processes of the same sort in the other. So far, we have the same situation as in the before mentioned splitting experiment where two heads were formed.

If we uphold the hypothesis of freely circulating inhibitory forces then how can we explain that two heads are regenerated? I think the proper answer is: the two halves are equally strong, therefore none can conquer the other, and therefore the party will be even, both will regenerate heads.

As a result of this conception and the fact that the head-producing capacity is greatest anteriorly this idea presents itself: by shortening one arm to give the other some predominance. Therefore a group of 18 animals had their left arm shortened by $^1/_3$, group *II* as shown in fig. 11. Another group of 22 had it shortened by $^2/_3$ (*III*). Those with both arms unshortened, 18 animals, were grouped under *I*.

To make sure that such lateral tissue which constitutes the arms is able to regenerate head, devoid as it is of median tissue in which the regeneration of heads normally starts, preliminary experiments were made without shortening the arms. The result showed that both arms were able to regenerate heads. But it is to be emphasized that they do so only after a length of time double that of a normal regeneration from an entire transversely cut surface.

Clear-cut as the idea in the experiment seems to be, it proved to be a complicated thing to carry through the experiment and many unforeseen situations occurred.

In the first place, in most animals the arms would coalesce soon after the operation. Repeated separating was necessary. Then the arms shortened and curved inward in such varied manners that the original scheme of the experiment was obscured. From these now distorted wounds blastemas often issued

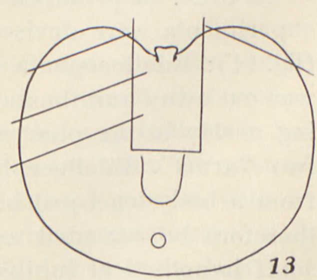

Fig. 13. *Bdellocephala.* Outline of the operations on the animal when in narcosis.

which differed much in size and position. Some of the animals lost their arms by basic constriction or they cytolized.

Notwithstanding these irregularities the main part of the experiments throw light upon our problem. The most common form of the course of the regeneration is that of fig. 12. It is here seen that the terminal wound is brought inward in continuation of the median wound.

In order to secure more terminal blastemas later series of experiments were made, where the cuts were laid obliquely as shown in fig. 13. In spite of this variation of the experimental procedure it was rather rare to see a terminal forwardly directed blastema. Therefore most of the heads which were regenerated had one eye made first, and always the eye lying nearest the front of the arm: a striking evidence for the stronger head-forming capacity of the anterior body region.

The result of the useful experiments may be summarized in this way:

		left	right
Group *I*	no head	8	7
	head	7	9
Group *II*	no head	11	5
	head	4	10
Group *III*	no head	14	6
	head	2	10

Before discussing these figures it must be born in mind that the right arms all are of the original length, they have not been shortened. They are therefore a sort of control for the power to regenerate heads at this rather anterior level of the body. After the previously found head-frequency curve of Bdellocephala the head-formation should in this place be somewhere in the neighbourhood of 95 $^0/_0$. But here only 62 $^0/_0$ are arrived at. I think that this result must be taken as a token of a feature which I have elsewhere (Brøndsted 1942) given account of: the eye-building force is at a maximum in the field, wherein the eyes themselves are lying in the animal and from here tapering towards all sides. It is therefore safe, I think, to conclude that the sides of the body in each transverse level have smaller eye-building capacity than the middle part in which the normal eyes are placed closely together.

But now as to our main problem: does the regeneration of eyes (heads) in the right arms inhibit the formation of eyes in the other arm, where on account of the forces underlying the head-frequency curve the eyes will normally be formed later?

A glance at the summary of the experimental results proves that this is not the case. Eyes are built also on the very short arms in group *III*. It may be asked if the two cases are samples in which no eyes have been formed in the right arm. But this is not so. In both cases beautiful heads have already been regenerated in the right arm.

If we now take the percentage of eye-formation in group *II* left arm, we find 27 $^0/_0$. Put into relation to the head-frequency curve there should have been something like 80 $^0/_0$. In group *III* left arm we find 13 $^0/_0$ corresponding to about 50 $^0/_0$ in the normal head-frequency curve at the corresponding level of the body.

The figures are admittedly too small to form any basis for elucidating the real power of forming heads in the sides of the body at this level. But a control experiment on 5 animals which had both right and left arm cut down to this level gave the result that no eyes were formed. Here no inhibition can be at work. I therefore think it safe to conclude that the lower figures of regenerated heads in group *II* and *III* are not due to any inhibiting force from the right arms but to a low ability in these parts of the body to regenerate heads at all.

Some of the unsuccessful experiments proved a very interesting fact: the shorter and longer arm of some of the operated animals succeeded in coalescing during the later part of the experimental period, and I let them have their way. Here the blastema of the shorter arm was built into the blastema of the longer, and a fine median head was the result of the cooperation. I think that we here once more have evidence of the strong regulating power and embryonic character of the blastema contrasting to the more rigid frame of the adult tissues. And here, in the blastema, it is therefore manifest that inhibitory processes are and must be at work.

Quite another question is this: when more points of the worm are damaged it is of course plain that the regenerative power of the entire worm is more stressed than when only one point is damaged. It therefore seems natural that the regeneration will proceed somewhat slower in a worm which has been severely operated on (as those described in these experiments) than in a worm which has not lost so much material, and which has not so big a wounded surface. This latter circumstance I lay some stress upon, and the fact has been dealt with in another paper (1942). The regeneration of any part of the body will to some extent retard the regeneration of any other body part. But this coarser physiological problem has of course nothing to do with the more subtile one here dealt with: a supposed inhibitance of the regeneration of structures of the same kind because they are of the same kind.

V.

The problem of the existence of inhibition in regeneration in planarians working through the adult tissues I have approached in quite another set of experiments, which were planned also to give answer to another question of some importance.

As far as I know all regeneration-experiments on planarians have dealt with wounds made so that a free outwardly directed surface of the body has been exposed, apart from such grafting experiments as those e. g. by SANTOS (1929) where holes were made to receive grafts.

I now put the question: if we take out some tissue in the centre of the body, if we so to speak make a window in the body, will the wound then be closed by a blastema regenerating the lost parts, or will, say, a head be regenerated in the window.

The result was rather a startling one: a head was in fact regenerated in several instances.

Big individuals of Bdellocephala were operated on and the experiment was carried out in this way: the animals were anaesthetized in nicotine. When they had duly contracted and were immobile a triangular or quadrangular hole was made with a fine knife given the form of a mortise-chisel. As in all my other experiments of this kind the operation was carried out on wax with sterilised instruments. The procedure is illustrated on fig. 14.

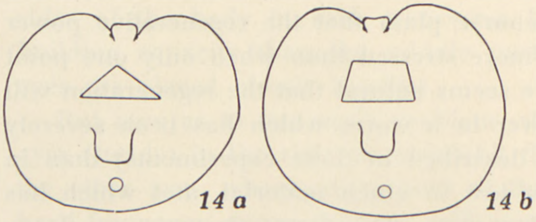

Fig. 14. *Bdellocephala.* Outlines of the anaesthetized animals with *a* triangular and *b* quadrangular window.

The operation was always made so that the caudal border of the window was lying well in the region of the animal which is able to regenerate a head when the animal is cut transversely at this level. The animals were placed in tap-water immediately after the operation.

In the following 3—4 days the wound must be continually cut open again because the animals will contract so that edges of the wound will glue together.

In the course of the experiment most of the animals were damaged in a rather discomforting way: when a Bdellocephala moves forward it will stretch its body, glue to the support with the underside of its head and then drag the body forward. This is sometimes a rather laborious task because the body itself may be pasted to the support by its own jelly. The head of the operated animal is connected with the body only by two comparatively thin bands of tissue on either side of the window, and these bands will therefore very often break, and so the animal is useless for the experiment. In order to avoid this calamity as much as possible there was nothing to be done except to keep the animals as long as possible in complete darkness, where their movements are nearly stopped. But the regular daily examination had to be done in light and then the mishaps took place. Therefore a large number of experiments had to be done in order to secure some results. 104 animals were operated on, but only 19 saw the whole game through, 4 of these had in the course of 22—31 days regenerated heads in the window, fig. 15.

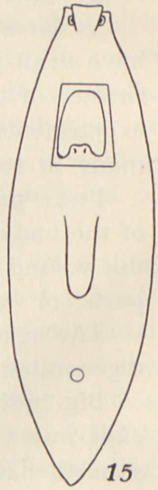

Fig. 15. *Bdellocephala.* Regeneration of a head in the window made as indicated in fig. 14*b*.

This head is formed by the blastema which had been built from the posterior border of the window. The other borders have their own blastemas without traces of eye-formation.

This experiment, I think, makes it difficult to uphold the rather cherished notion of a "Ganzheitsfaktor" working in the bodies of multicellular animals. It also makes it difficult to uphold the more comprehensible conception of the existence of an inhibiting

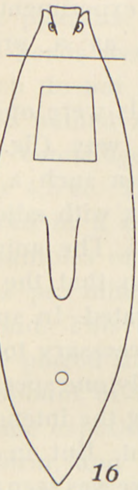

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Fig. 16. *Bdellocephala.* Besides the making of a window a transverse cut separated the head from the body.

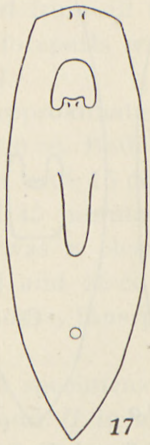

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Fig. 17. *Bdellocephala.* Regeneration of a head in the window in spite of the regeneration of a head from the transverse cut anteriorly as indicated in fig. 16.

factor emanating from the already existing head and working freely in the tissues.

Now the possibility remains that a head in process of formation from a more anteriorly laid cut may inhibit the formation of a head in the window. It is possible that inhibiting forces are regenerated in a blastema in which head-formation is going on in the form of substances circulating freely in the body.

To test this possibility several of the animals with windows were cut transversely as indicated in fig. 16. Only a few specimens came through safely, but two of these had formed heads in the window besides a big head regenerated from the anterior cut surface, fig. 17. This experiment also rejects the assumption that inhibiting forces or inhibiting substances generated in a head-forming blastema were able to inhibit the formation of heads elsewhere in the body.

VI.

The experiments just related were made with a new technique which seems to open new possibilities in studying the formbuilding potencies of the planarian tissues.

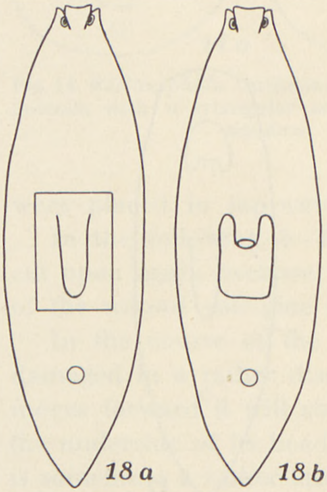

Fig. 18. *Bdellocephala*. *a* the operation: the pharynx with the adjoining tissues is cut away so producing a window. *b* the result: a new pharynx stretching freely into the window is regenerated.

The following experiment deserves a short account as a preliminary statement.

Several animals were operated on in the following way (fig. 18 *a*): a window was cut in such a way that the whole pharynx with adjoining tissues was removed. The animals contracted strongly so that the windows were quite obliterated. In spite of repeated cutting—necessary to hold the window open—only one specimen succeeded in fulfilling the intended scope of the experiment. But in return a very striking feature was seen (fig. 18 *b*): a new pharynx had been regenerated, and this pharynx was nakedly protruding into the empty window. So this experiment gives evidence that an organ may be formed isolated without the co-regeneration of the usual adjoining tissues. This experiment is now to be repeated on a bigger scale and a microscopical investigation made. This is worth while because it seems to point in the direction of a certain and very curious self-differentiation of the planarian pharynx.

VII.

I should like here to take the opportunity to relate a few experiments concerning the question about the possible existence of organizing substances in the planarian body.

It has been stated (Brøndsted 1939) that only the part of Bdellocephala lying anteriorly to the pharynx is able to regenerate a head. Although the experiments in the paper cited and those related in the paper in hand strongly point in the direction, that the organizing powers are bound up with the living cells, the possibility is still open that organizing substances may exist independently in the body fluids.

To try this possibility the following experiments were made.

190 specimens of Bdellocephala were transversely cut just before the pharynx. All the fore-parts possessing the ability to regenerate heads were treated together apart from all the hind-parts which do not have this ability. The foreparts will henceforward be named *I* and the hind-parts *II*.

I were centrifuged to determine the approximate volume, which was 4,5 cc. The volume of *II* was 7,0 cc. Both *I* and *II* were ground in a mortar with glasspowder and 15 cc alcohol. The two samples of gruel were centrifuged 45 minutes at 2500 revolutions pr. minute. Over the residue was a clear yellow-reddish fluid. This fluid was 10 cc from *I* and 15 cc from *II*. They were placed in an exsiccator for 24 hours, thus producing a small amount of a yellow-reddish paste.

The same experiment was done with 100 specimens *I* and *II* in chloroform, and with 100 specimens *I* and *II* in acetone.

Now the hind-parts of several Bdellocephala were cut from the bodies just behind the genital pore. Several others were cut between the genital pore and the pharynx. The anterior cut surfaces of some of each series were smeared with paste *I* and some with paste *II* of each of the three solutions. In the same way some were smeared with the residue of *I* and *II* from each series. All the specimens were laid on silk-gauze suspended on Schotté-tables (Brøndsted 1939). Here they remained for 48 hours, then they were placed in water. Several died, but in all others, where regeneration took place, no head-formation occurred.

The only thing these crude experiments tell is that they do not sustain the hypothesis about head-organizing substances circulating freely in the planarian body.

VIII. Conclusions.

From the foregoing and from my papers 1939 and 1942 may be seen that two cardinal problems in regeneration have been the main topic of my investigations: organizing forces and inhibiting forces.

In surveying the vast literature upon Planarian regeneration it is a striking fact that so many contradictory statements have been made. It is not the aim of this paper to reconcile these statements. Many more experiments are needed to do that. But I venture to think that now a certain general scheme in these obscure matters is beginning to reveal itself. The scheme may briefly be formulated thus:

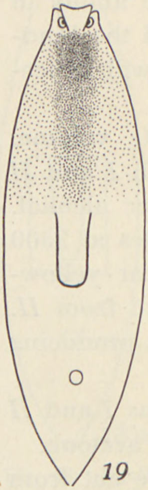

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Fig. 19. *Bdellocephala.* The headproducing field. The capacity for regenerating head is strongest where the dots are lying most densely, from here tapering both towards the sides and towards the pharynx.

1. The regeneration (organizing) powers for building up the various organs are unevenly distributed throughout the planarian body. The evidence for this is plainly exhibited in the head-frequency curve. This distribution is fixed for every species.

2. The conception of "fields" so brilliantly set forth by Huxley & de Beer in their book "The Elements of Experimental Embryology", 1934, has a very definite meaning in the planarian body. There exists f. i. a distinct head-forming field of larger or smaller extension in the various species. The field has a center of maximum activity, from here tapering unevenly towards all sides (fig. 19, and Brøndsted 1942).

3. The field is already determined in the embryonic state of the animal: the head-frequency curve of the newly hatched Bdellocephala is the same as that in the adult.

4. The organizing powers of the field is associated with cells lying in the field and therefore manifests itself in the regeneration blastema.

5. The development of the blastema is at the beginning seemingly broadly determined by the body. But looking more closely into the matter it is seen that this "determination" is not to be understood as an undefinable power emanating from the body but as a more definite matter: the cut has set free to work the already determined cells lying in the tissue on the place, where the cut has hit the cells. Here we meet the conception of Goetsch 1932, who claims that there are head- and tailbuilding cells in the planarian body. Here also the idea of Child about a physiological dominant region comes in.

When a cut is made and, say, head forming cells among others are set to work, these cells take the lead and by and by organize the other cells in the blastema. There are potencies for more than one head in the very young blastema (by separating the blastema in two or more distinct areas as in LUS's experiments, many heads may be formed), but in the undivided blastema inhibiting as well as organizing forces are transmitted from cell to cell just as in the young embryo, and so a harmonious result of the regeneration processes is arrived at. That this is so depends on one thing, namely that a formbuilding gradient is set up at once after the wound is made. Let us consider Bdellocephala. The head-building potencies, the head-building field as we may call it, has a form and an extension probably as that depicted in fig. 19. Cells responding to a call for regeneration of a head are lying most densely in the anterior middle portion of the animal, from here tapering towards all sides. It will be hard to make a wound in the forepart of the animal where a gradient for head-building forces will not at once display itself. As soon as the gradient goes to work, the result must be harmonious. If the gradient is not set up at once two or more head-building centres may start simultaneously and as many heads or at least rudiments of heads will be formed.

I think that this working hypothesis will solve many of the difficulties associated with regeneration problems in planarians and perhaps elsewhere. But many more experiments are necessary to establish the idea on firm ground.

Summary.

1. The head of Bdellocephala was removed with a transverse cut. A tail was transplanted to the wound. No head is regenerated. Fig. 1.

2. The same experiment was done but besides a cut was made on the side of the body just caudad to the transplanted tail. The removed head was transplanted to this wound. Fig. 2. The head will not develop into the "working" head of the animal. Fig. 3. If the tail is removed a new head will regenerate anteriorly despite of the old head.

3. A large number of Bdellocephala were transversely cut at various distance between head and pharynx. Fig. 4. In all cases new regeneration tissue was intercalated between the new head and the old tissues, so that no morphallaxis took place in this latter. Fig. 5.

4. A section of the forepart of Bdellocephala was cut away by a transverse cut and the head grafted upon the thus shortened body. Fig. 6. New regeneration tissue was intercalated between head and body, so restoring the normal relative length of the animal. Fig. 7.

5. Light and dark coloured animals were divided in halves by longitudinal median cuts. Fig. 8*a*, *b*. A light and a dark half were grafted together so forming a chimera the two halves of which were easily recognizable. Fig. 8*c*, *d*. The heads were removed by a transverse cut. A new head was regenerated, common for both halves. Fig. 9.

6. The same experiment was done so that the amount of tissue of the two partners of the chimera were differing much in volume. Fig. 10*a*. The result was however the same: a new median common head was regenerated showing no asymmetry. Fig. 10*b*.

7. The median portion with head of Bdellocephala was removed so that two "arms" remained. Fig. 11. Heads may be regenerated upon both arms regardless of different length of the arms. So no force will travel from one arm to another to inhibit head-building there.

8. A window was made in the fore-part of Bdellocephala. Fig. 14. A head may be regenerated from the caudal border of this window as well in the presence of the old head, fig. 15, as during regeneration of a new head instead of the old one removed. Fig. 17.

9. A preliminary statement of another experiment with this new technique is given: the pharynx with surrounding tissues is removed leaving a window open. Fig. 18*a*. Into this window a pharynx without surrounding tissues is regenerating. Fig. 18*b*.

10. Extracts from foreparts of Bdellocephala (which alone are able to regenerate heads) in chloroform, alcohol and acetone are not able to induce head-regeneration in hind-parts of the worm, neither are the residues.

11. From all the experiments the conclusion is drawn that during regeneration organizing power is transmitted from cell to cell and is not due to freely circulating "organisatorstoffe". So also with the conception of inhibiting forces or substances during formbuilding processes. The experiments sustain the hypothesis that inhibiting forces are also transmitted from cell to cell in the regeneration blastema.

12. A preliminary working hypothesis for a comprehensive understanding of regeneration—especially in planarians—is set forth.

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