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REGENERATION IN PLANARIANS INVESTIGATED WITH A NEW TRANSPLANTATION TECHNIQUE

BY

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I. Does the Head of the fully differentiated, adult Planaria act as an Organiser? Experiments on *Planaria lugubris* O. Schm.

It is a question of considerable importance whether the fully differentiated, adult head of a planaria can set up new gradient-fields in the grown-up body when implanted into different parts of an adult planaria; that is to say, whether the head contains agents which are able to overthrow the already stabilised polarity of the fully differentiated body.

If this should prove to be the case, the planarian head would be comparable to the amphibian organiser, and the tissues in the body to the ventral epidermis in the gastrula-stage. The whole system of the grown-up planaria would then in a sense be embryonic in character.

The question has been dealt with, or at least touched upon, by RAND and BROWN (1926), SANTOS (1926), STEINMANN (1933), OKADA and SUGINO (1934), SPIRITO (1935). The results have been rather contradictory. The possible causes of the discrepancy will be discussed after the exposition af my own results.

It is quite clear that transplantations on the highly regenerative planarians may be expected to yield valuable information about fundamental questions in the whole problem of regeneration. Many authors have therefore developed trans-

plantation techniques, thus, besides the above-mentioned, T. H. Morgan (1900), L. V. Morgan (1906), Goetsch (1921), Li (1928).

The techniques may be grouped under two headings. In the one kind the pieces are held together cut face to cut face by glass, collodium, paper and the like. In the other, small pieces are inserted into holes made in the body. The method of Okada and Sugino is unfortunately not stated in their paper. The other methods seem to be rather troublesome. It was therefore to be expected that a technique which would be able to yield convenient and expeditious work would prove to be of great advantage for the solving of many questions in the problems of regeneration in planarians.

The following principles were applied in the development of a suitable technique.

1. Spontaneous muscle-contractions during, and a suitable time after, the transplantation must be prevented. A convenient narcotic had therefore to be found. Chloretone, which is in general use for planarians, proved useless, because the muscles are often in chloretone-narcosis in such a state of tension that the form of the animal is somewhat contorted, and this then prevents a systematical transplantation, inasmuch as the cut surfaces will not fit one another. It is true that in a heavier narcosis the muscles will be more relaxed, but in that state the animal is generally not able to live long enough to afford time for the cut surfaces to grow together. The results of experiments with various other narcotics was that nicotine proved to meet the requirement of a suitable relaxation of the muscles together with a non-impairment of the viability during the narcosis.

2. Experience has shown that living pieces of planarians are almost motionless in darkness until an advanced regeneration has taken place. But even if the transplanted pieces were both narcotised and placed in the dark, they did not coalesce well when placed in water. It was therefore necessary to develop a method whereby the pieces would retain their viability without water as the surrounding medium, but with moisture enough to prevent a drying of this rather delicate kind of tissues. The pieces were placed on silk extended over small frames of glass. SCHOTTÉ (1930) devised a method for operating on Anuran eggs. He used small glass-rings with silk extended over them. These Schotté-tables proved to be excellent.

The new technique was developed on *Planaria lugubris*. This species is known to possess a formidable resistance towards operations and a power of regeneration almost unique even among planarians.

After a good many experiments the following rather simple technique was developed.

The animals are taken directly from the aquaria and put into petri dishes with tap-water containing about 0,001% nicotine. This concentration may vary a great deal. It is convenient to have 50 cc water in the dish and pour 5—15 drops of a 1% nicotine-solution into it. After about one hour the operations can be performed. The cutting is done by means of small scalpels sterilised in the gas-flame before every cut. The operations are performed on wax or a mixture of wax and paraffin. It is most convenient to cast the wax in a 5×2 cm petri dish, and cool quickly so that the block may easily be taken out. Before every operation the surface of the block is heated in the flame.

With a pincet the cut pieces are placed on the Schotté-tables. These have previously been sterilised and covered with sterilised silk which is fastened under the collar of the glass-ring with a likewise sterilised caoutchouc ring. The Schotté-tables are placed in petri dishes, e. g. 10×2 cm, in which is placed nicotine-water of the same concentration as that used for the narcotising of the animals. To avoid troublesome air-bubbles beneath the silk, the nicotine-water is pipetted over the silk so that this is moistened and the water oozes down into the dish from under the Schotté-tables. In order that the silk may be easily moistened it has to be carefully washed before use in pure soap, rinsed several times in tap-water, then in distilled water, after which procedure it can be stored in 70 % alcohol. Before use it has of course to be thoroughly rinsed in distilled water.

When the cut pieces of the planarians are put into their proper place on the silk, a few drops of the nicotine-water are pipetted over them in order to clear away loose cells and the like. With a flamed pincet and a glass-rod with a globe (according to Spemann) the pieces are now pushed close together. The cover of the petri dish is put on in order to prevent evaporation, and a cover of pasteboard is placed over the dish in order to make the moist chamber dark. This must be done after every transplantation. A few pieces can be placed on every Scotté-table, and several tables in every dish. When the desired transplantations have been made, the dish should be put in a dark and cool room. A room or ice-box of 5—10° C. has proved most useful. If some of the pieces have moved apart during transport they may be replaced.

If healthy animals are used, especially if they have only

been in the laboratory for a few days after capture (they are not to be fed), up to 100% of the transplantations may give useful results.

After 24 hours the pieces of the animals will adhere so closely that they can be put into pure tap-water. This is best done in the following way: the Schotté-tables are put in a petri dish with tap-water reaching half way up to the silk with the animals. Then more water is very cautiously poured into the dish so that it will slowly cover the silk itself. In most cases the animals will separate from the silk, and the table can be removed without touching them. If not, they can be removed with the glass-rod.

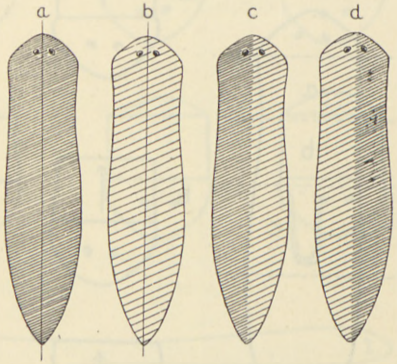


Fig. 1. *Planaria lugubris*. a, dark- coloured, b, light-coloured sample. The longitudinal lines indicate the plane of cutting. c and d, light and dark-coloured halves from a and b grafted together to form chimaeras.

After this procedure the dish should again be placed in the dark, but it is not necessary to put it in the ice-box. After another 24 hours, the pieces have grown so closely together that they may be handled without further caution.

The effectiveness of the method will be seen from fig. 1, where the longitudinal half of a dark animal has been transplanted to a light-coloured one.

With this method the first question put in this paper can be investigated: can the fully developed head of a planarian set up new gradient-fields in the grown up body when implanted elsewhere?

These experiments were also made upon *Planaria lugubris.*

In order to avoid the formation of regeneration-blastemes which would only obscure the results, the cuts were made

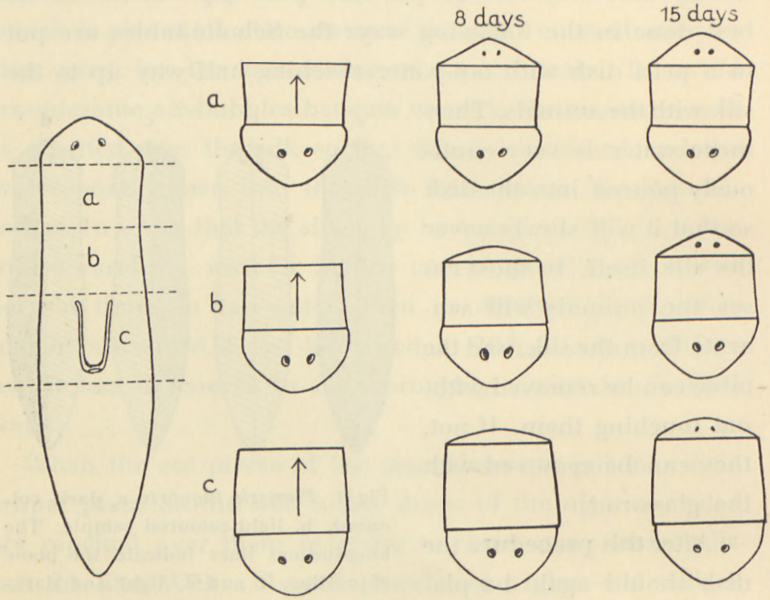


Fig. 2. *Planaria lugubris.* Exper. I. The dotted lines indicate the cuts made. The arrows indicate the polarity of the segment. The transplanted heads are pointing downward.

in such a way that the faces which were to be placed against one another were of the same size. All the transplantations were auto-transplantations.

Experiment I. This was conducted in three series (fig. 2), in order to test the question at different levels of the body. 5 transplantations were made in each series. After 8 days the body segments in the a-series had built blastemes with eyes in accordance with its own polarity. The same

situation only occured after 15 days in the b- and c-series. After this time several animals had died, only 4, 3 and 1 chimaeras remaining in the series a, b, c respectively.

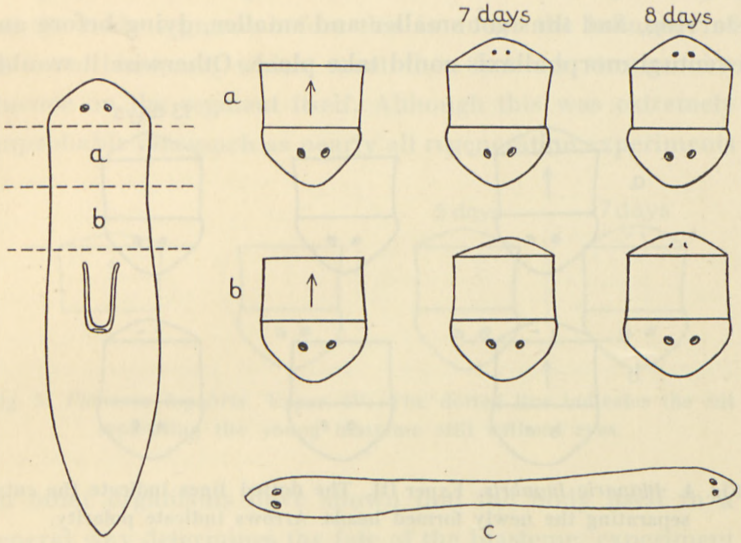


Fig. 3. *Planaria lugubris.* Exper. II. a and b as in fig. 2. c, chimaera formed by the old head transplanted to an a-piece, which has formed its own head according to its own polarity. The two animals are dragging one another out into a long shape by their efforts to go forward.

Experiment II. Essentially the same experiment as I: series a and b (fig. 3). After 7 and 8 days all body segments had developed heads in their blastemes. Of the 15+15 transplantations 10+8 chimaeras were now living.

Experiments I and II prove that the fully formed head is not able to prevent the formation of a head in the blasteme of the body segment into which it is grafted in the opposite direction of the antero-posterior polarity. Neither is it able to revert the neuro-muscular movements of the body-segment. Chimaeras are formed, animals with a head at

each end; and these two heads strive to get away from one another, thereby dragging the whole thing out into a rather long thin thread (fig. 3 c). As the animals had no pharynges, they were not able to take food, therefore they were starving, and thus got smaller and smaller, dying before an eventual morphallaxis could take place. Otherwise it would

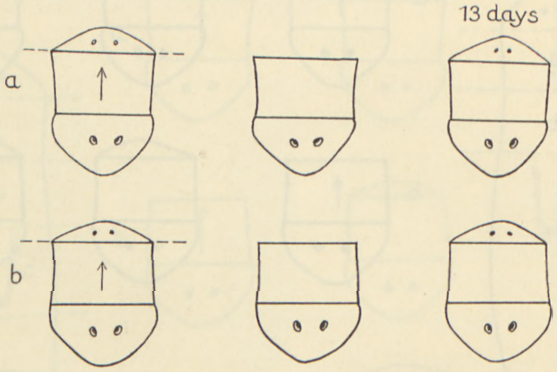


Fig. 4. *Planaria lugubris*. Exper III. The dotted lines indicate the cuts separating the newly formed heads. Arrows indicate polarity.

of course have been of considerable interest to see if the morphallaxis would proceed in the same order in the animal with the adult head and that with the head being built in the blasteme. In the future I hope I shall be able to look into this theoretically important question, using larger animals.

The likewise interesting question as to the histological connection between the two animals is also postponed.

Experiment III. As we have seen, new heads are formed in the blasteme of the body-segments. After 15 days these heads are not yet fully differentiated and pigmented. Now it might be argued that, if this new head were cut away, the grafted head might perhaps, so to speak, have a chance. This experiment (fig. 4) was made with three chimaeras of series a and b of experiment II. After

13 days all six animals had built heads in the blasteme formed on the newly cut front-surfaces of the body-segments.

Experiment IV. But perhaps an organising agent from the grafted head is present but has not had time to work on the body segment before this is under the influence of its own blasteme, which must then have a stronger influence on the segment itself. Although this was extremely improbable inasmuch as nearly all regeneration experiments

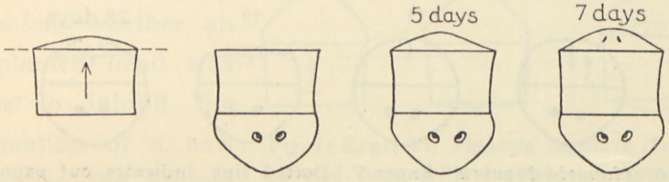


Fig. 5. *Planaria lugubris*. Exper. IV. The dotted line indicates the cut separating the young blasteme still without eyes.

on other organisms have shown that the body itself in a general way determines the fate of the blasteme, experiment IV was carried out.

To investigate this question the blasteme on 4 pieces with blastemes in which no traces of eyes were as yet to be discerned, were cut away. Then a head was grafted. But after 5—7 days new blastemes were formed with eyes in them (fig. 5).

Experiment V. Finally it may be argued that even the very first beginning of the new blasteme is able to resist an influence from the grafted head. In order to test this I proceeded as follows.

Not rarely it happens that a cut surface contracts from side to side so that the wound is closed in a median suture. If this happens, we get after the transplantation a curious sort of chimaera, consisting of a head and a piece of a body which do not build any blasteme and therefore

do not develop any further. Analogous facts are known from other experiments and are of considerable interest. But no further stress will be laid on this here.

Here we have body-segments which cannot have been influenced by any blasteme. Here, then, is a possibility for the head to exert its influence on the body-segment. And in order that this possible agency might have sufficient time to work, I waited 11 days before cutting away the closed

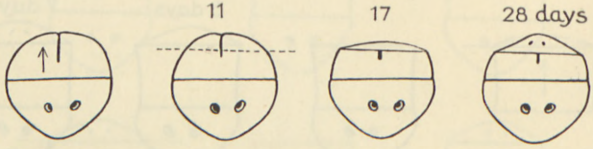


Fig. 6. *Planaria lugubris*. Exper V. Dotted line indicates cut exposing a free surface. Arrows indicate polarity. The dark line medially in the upper segment indicates suture of wound closure.

wound, so that a fresh cut surface was exposed. Blastemes were formed and they built heads. 4 samples were treated in this way, all showed the same result (fig. 6).

Experiment VI. The five foregoing experiments were conducted in such a way that a possible power of the head to reverse the antero-posterior axis was tested. After the negative results it did not seem likely that the head would be able to reverse the dorso-ventral axis. The experiment, however, was made. Three series were done, corresponding to the series in experiment I. 5 transplantations were made in each series. Only three transplantations from the a-series were successful. After 10 days the result was that on the only two now living chimaeras small blastemes had grown out from the body-segments forming heads with the dorsal side in accordance with the body-segment, and not with the grafted head (fig. 7).

Discussion.

Now two questions will at once arise. 1. What importance can be attached to these results? 2. How can the disagreement with the findings especially of SANTOS (1929) be explained?

In order to discuss these questions it is necessary to review the literature which touches upon these problems.

RAND and BROWN (1926) had 8 successful transplantations on testing the problem whether an implanted head was able to inhibit the formation of a new head in the animal into which the head was grafted. The head of the animal itself was removed after the grafting. In the two experiments new heads were built from the animal despite the implanted head, although the blastemes were removed several times. In two other experiments the blastemes were removed so close to the implanted head that after a time this became the head of the animal. In the other experiments the head of the animal was severed close to that of the graft, and in those cases the graft became the head of the animal.

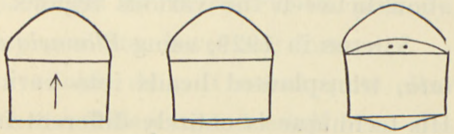


Fig. 7. Exper VI. *Planaria lugubris*. Transplanted head with ventral side upwards, no eyes are therefore seen. Arrow indicates polarity.

It is quite clear that we have here to deal with another problem than that with which we are here concerned. RAND and BROWN's experiments do not involve the problem of reversing the polarity, though there is some similarity in so far as RAND and BROWN deal with the question whether a new, implanted head may be able to replace the original head of a body-segment. And this has been proved to be

the case if only the implanted head comes to lie in the axis of the body into which it is transplanted. This is apparently the case in the experiments of Rand and Brown. So far there can be no question of an organising effect of the head as such. It is to be expected that a sort of reorganisation of the whole chimaera will thereafter take place. But we do not know from what part this reorganisation is started; most probably it takes place by a collaboration between the various regions of the body.

Santos in 1929, using *Planaria dorotocephala* and *maculata*, transplanted heads into various levels of the body. His technique is entirely different from that here recorded. Santos cuts out the part of the head containing the head-ganglion. This piece is inserted into the body in which a corresponding hole, triangular or round, has been made to receive the graft. Santos found that such a graft is capable of reversing the polarity if transplanted post-pharyngeally.

On comparing the findings of Santos with those recorded in this paper it must be emphasised that the techniques employed are entirely different. And this may be the cause of our different results. It is not clearly to be seen, but it seems most probable, that a certain amount of regeneration-blasteme is formed in the place where the head has been grafted. If this is the case, a new set of gradients are undoubtedly set up under the influence of this blasteme. A further complication as to the interpretation of the results of Santos is that *Planaria dorotocephala* at any rate is not the appropriate material to test these questions, because this species forms new heads by spontaneous fission post-pharyngeally.

Steinmann (1933) on *Planaria gonocephala* seems to

have used a similar technique and obtained similar results. But here also the formation of blastemes is likely to have taken place.

Okada and Sugino (1934) do not find any disturbance of the orientation of the host after the implantation of heads. Unfortunately it is not stated how the experiments were carried out.

Spirito (1935) using *Planaria torva* finds that foreparts implanted into the genital tracts do not reserve the polarity, but supplementary pharyngeal structures are formed.

Considering all the available data, it seems quite clear to me that in planarians the differentiated adult head has not the power to organise a grown-up body segment, at least not into the reversal of its polarity. To do this a blasteme has first to be formed. That this is the case has been proved various times, most extensively by Kahl (1935).

It would seem, therefore, that there exists a fundamental difference between the fully differentiated head and the head under differentiation in respect of organising powers.

If this is so, it would be a fact of considerable importance. It would show that only if planarian tissue has the opportunity of forming blastemes, that is, undifferentiated tissue, is it able to organise.

The blastemes would then resemble embryonic tissue in two respects: 1. it differentiates and 2. it influences by a kind of organisation-proces other tissues, and remoulds them.

So far I have not been able to transplant pure blastemes to try their effect in this respect, but all regeneration-experiments on planarians seem to show that this is the case.

Summary.

A new transplantation-technique on planarians is described.

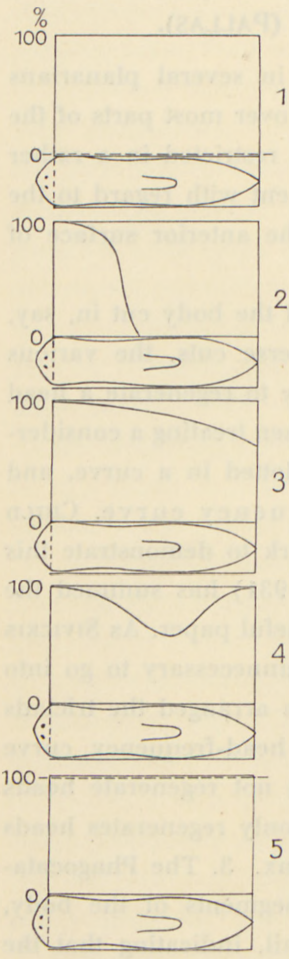
With this technique it is shown that the fully differentiated grown-up planarian head does not act as an organiser when transplanted to other parts of the planarian body with reversed polarity.

II. The Head-Frequency of the Fresh-Water Planarian *Bdellocephala* punctata (PALLAS).

Whereas the regenerative power in several planarians seems to be fairly evenly distributed over most parts of the body, this power is in some species restricted in a rather peculiar way. This is especially evident with regard to the ability to regenerate a head from the anterior surface of transversely cut pieces.

If a planarian be decapitated and the body cut in, say, 6 pieces of equal length by transverse cuts, the various segments will show a different ability to regenerate a head at its anterior surface. The results, when treating a considerable number of animals, may be plotted in a curve, and this curve is termed the headfrequency curve. CHILD and his school have done much work to demonstrate this curve in several species. SIVICKIS (1931) has summed the results up in a very suggestive and useful paper. As SIVICKIS gives a complete bibliography it is unnecessary to go into details. Suffice it to say that SIVICKIS arranged the triclads into 5 groups with regard to the head-frequency curve (fig. 8). 1. The Belloura-group does not regenerate heads at all. 2. The Dendrocoelum-group only regenerates heads from segments anterior to the pharynx. 3. The Phagocata-group regenerates heads from all segments of the body, but the curve slopes towards the tail, indicating that the

ability to regenerate heads from posterior segments is not always present. 4. In the Planaria dorotocephala-group, in which there is a fall in the curve a little beyond the middle of the body, the hind part regenerates heads quite as well as the forepart. It must be remembered that Planaria dorotocephala is able to develop heads spontaneously in the hind part of the body, in this way multiplying by natural fission. 5. In the Planaria velata-group every part of the body is able to produce heads with equal facility. The head-frequency curve is here constant.



The difference in the ability to regenerate heads in planarian species which are of about the same organisation grade is a warning against the too rigid generalisation of the current hypothesis that regenerative power is correlated with and proportionate to the degree of organisation.

It is obvious that the feature is

Fig. 8. Diagram of head-frequency curves of planarians after the grouping of Siwickis (1931). Dotted lines across the heads indicate the cuts separating the heads from the body. The curves indicate the ability of a cut surface, corresponding to each point of the curve, to regenerate a head. 1, the Belloura-group. 2, the Dendrocoelum-group. 3, The Phagocata-group. 4, The Planaria dorotocephala-group. 5, the Planaria velata-group. Dotted lines in 1 and 5 indicate absent (1) and absolute (5) power of regenerating heads.

of fundamental significance for our understanding of regeneration in general. The question is so important because it is likely to throw light upon the problem why some animals regenerate easely, others with difficulty or not at all. But this will not be discussed in this first paper in a series of studies on the phenomenon.

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With regard to other problems of significance for the understanding of regeneration on which I am engaged it was necessary to find a planarian species, the head-frequency of which was of about the same nature as that of the Dendrocoelum-group.

The problem to be solved had to be investigated by means of the transplantation method described before. Dendrocoelum proved unsuited for the purpose, because this animal is very active and had to be narcotised so heavily in order to be kept immovable sufficiently long that too many of the animals died after the operation.

One of the largest species of the European freshwater planarians is *Bdellocephala punctata* (PALLAS). It often attains a length of 30 mm. and a breadth of 5—7 mm. On account of its relatively slow movements, its size and its tolerance of operations this species proved very useful for a larger series of planned experiments. Since the head-frequency curve enters as an important element into the questions investigated in some of the experiments, it was necessary to find out this curve for the species.

The animals were taken in the freshwater-lake, Furesøen, near Copenhagen. Beneath stones by the shore Bdellocephala can be obtained in sufficient numbers throughout the summer and autumn, and if the winter is

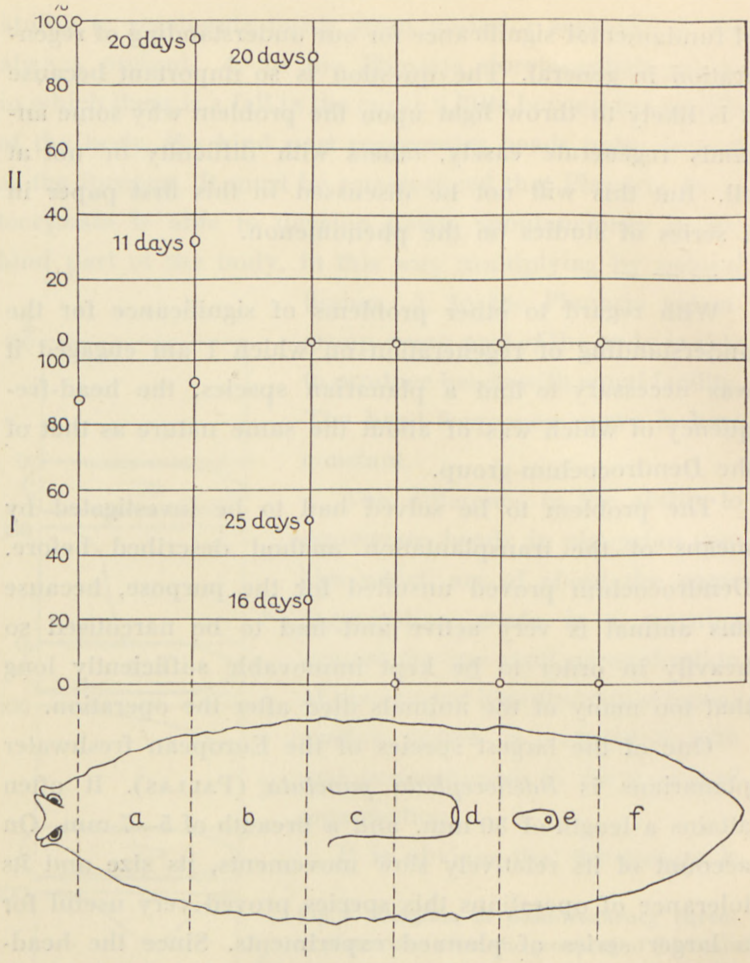


Fig. 9. *Bdellocephala punctata.* Dotted lines indicate cuts made transversely to test the power of various levels of the body to regenerate heads. In I (exper. 258) 86 % of the *a*-segments had regenerated heads after 16 days, the *b*-segments 92 %, the *c*-segments 26 % after 16 days, 52 % after 25 days. The d—f segments did not regenerate heads at all. In II (exper. 259 b) all *a*-segments had regenerated heads after 11 days, the *b*-segments 32 % after 11 days, 94 % after 20 days. The *c*-segments 0 % after 11 days, 89 % after 20 days. d—f segments did not regenerate heads.

mild enough big samples may be collected in March, most of them in process of laying cocoons.

Freshly collected animals were decapitated, the body cut in 6 segments by transverse cuts as indicated in fig. 9 (dotted lines). The result was the same with 20 animals which had been kept starving in the laboratory for a fortnight, and likewise with animals starving for a month or more.

All experiments showed that the ability to regenerate a head was lost in the body at a level just before the pharynx. Fig. 9 gives the result of two experiments, 258 and 259b from the protocol (20 animals in each experiment).

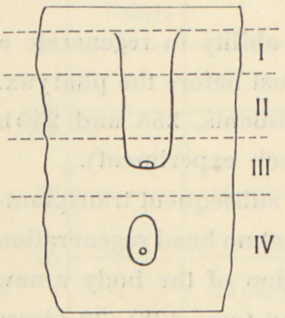
As it was of vital importance for subsequent transplantation experiments to be quite sure that no head regeneration occurred behind the pharyngeal region of the body a new series of experiments were carried out (exp. 428). 20 pieces of Bdellocephala comprising the body just before the beginning of the pharynx to the region behind the genital pore were divided by transverse cuts as indicated in fig. 10, that is: group I had its anterior surface just before the beginning of the pharynx, group II a little behind the beginning of the pharynx, group III just before the mouth, group IV between the mouth and the genital pore. No one of the four groups showed any sign of a regeneration of heads after 20 days, nor later. After the lapse of 20 days all pieces in the fore-region of the body had regenerated heads.

In order to be quite certain that the tail region around the genital pore is also unable to develop heads, a series of experiments were made as indicated in fig. 11 (protocol exp. 430): The cuts were laid just anterior to, II just posterior to the genital pore. No head formation took place.

This makes it certain that the head-frequency curve in Bdellocephala is of the same type as in Dendrocoelum, that

is Sivickis group II. The curve may be drawn as in fig. 12 (full line).

Before closing this brief report I wish to emphasise that the inability to regenerate heads in the hind part of the



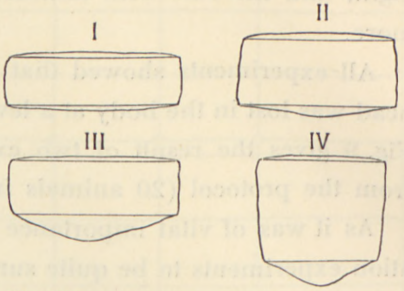
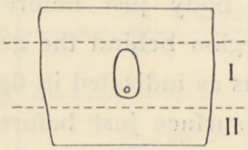


Fig. 10.



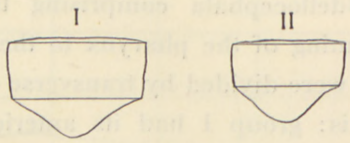


Fig. 11.

Fig. 10. *Bdellocephala.* Dotted lines indicate cuts separating the middle body in segments I—IV. After 18 days no trace of head-formation. Note greater tail-regeneration in IV. — Fig. 11. *Bdellocephala.* The tract around the genital pore. Dotted lines indicate cuts separating segments I and II. No head-formation. Note well regenerated tails.

body is by no means equivalent to loss of regenerative power. On the contrary, the ability to regenerate tails on the hind surface of a segment of the body is undoubtedly greater in segments from the posterior parts of the body (fig. 10, 11).

Whereas it is easy to ascertain the regeneration of heads because of the occurrence of pigmented eyespots, it is much more difficult to tell when a tail is regenerated. This is so

because an undifferentiated regeneration blasteme may easily be confounded with the earlier stages of a tail in regeneration. A sure sign of the regeneration of a tail is that branches of the intestine grow into the blasteme. Using this criterion I have been able with some certainty to ascertain

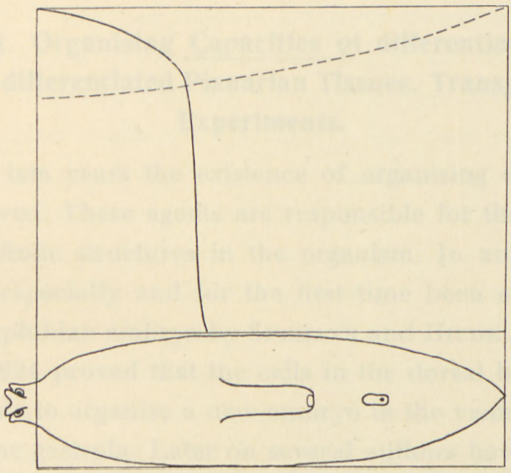


Fig. 12. *Bdellocephala.* Curve in full line: head-frequency curve. Dotted line: approximate tail-frequency curve.

that the ability to regenerate a tail is more pronounced in the posterior segments of the body than in the anterior segments. With some certainty the tail-frequency curve of Bdellocephala may therefore be depicted as in fig. 12 (dotted line).

It may be added that this observation in some measure sustains the hypothesis that in the planarian body there exists two gradients, one for the formation of the head and foreparts, and one for the formation of the tail and hindparts, the first generally sloping from head to tail, the other from tail to head. It is impossible to resists the suggestion

that the results reported here present a striking analogy to the gradient in eggs, e. g. the animal and vegetative gradient in the see-urchin egg as suggested by Runnström (1928) and borne out by him and his school (see e. g. Hörstadius 1935 and Lindahl 1936).

III. Organising Capacities of differentiated and non-differentiated Planarian Tissues. Transplantation Experiments.

1. Of late years the existence of organising agents has been proved. These agents are responsible for the building up of definite structures in the organism. In animals this fact has especially and for the first time been established in the amphibian embryo by SPEMANN and HILDE MANGOLD, who in 1924 proved that the cells in the dorsal blastopore-lip are able to organise a new embryo in the ventral epithelium of the gastrula. Later on several authors have contributed studies on the question (For literature concerning the amphibian embryo see BRØNDSTED 1936. For literature on the whole question of organisation, especially from the biochemical point of view, see J. NEEDHAM 1938).

The question of the nature of the organising agents has for the present come to a standstill. This is because it has been made clear that in all true organising agents we have to reckon not only with trigger-substances from the "organiser", but with agents generated in the reacting cells themselves. And this makes the problem so complicated and intricate that we have to wait for a closer examination of the finest metabolic processes in the cells under organisation before we can advance any further with regard to the chemical nature of the organising substances. In the mean

time it will be well to collect more facts concerning organisation processes on the biological level of research.

2. The processes in tissues under regeneration which have to do with the moulding of the tissues into the shape of the lost parts are directly comparable with processes in embryonic development.

Much evidence has been brought forward in support of the idea that the regeneration blasteme in later stages gives off substances which organise not only the blasteme's own tissues but may also reorganise the whole body. Thus a new head regenerating from a piece of a planarian is able to remould this piece to a complete worm: the well known phenomenon, called by Morgan morphallaxis. Here the head in regeneration is a true organiser. And it is quite conceivable that this influence of the head in the state of regeneration on the body is exercised by means of organising substances.

3. Polarity exists in almost every organism. The polarity of a body displays itself as a dynamic force when regeneration takes place, in so far as the polarity, and therefore the basic shape, of the regenerated bud is determined by the body. We do not know in what way this determination takes place. It may be by diffusing substances or by cells predetermined for the special task. The question therefore arises if this displaying of polarity when a blasteme is stamped is comparable with, say, the displaying of the forces in the blastopore lip. If so, it would be probable that dynamic polarity were due to organising substances.

4. In order to penetrate a little into these obscure problems the following question was raised: If a segment from the fore-part of a planarian which is able to regenerate a head from cuts in the fore-part is transplanted with reversed

polarity to a segment of the hind-part which is not able to regenerate a head, will the transplanted piece in some way induce the hind-part to regenerate a head, when the

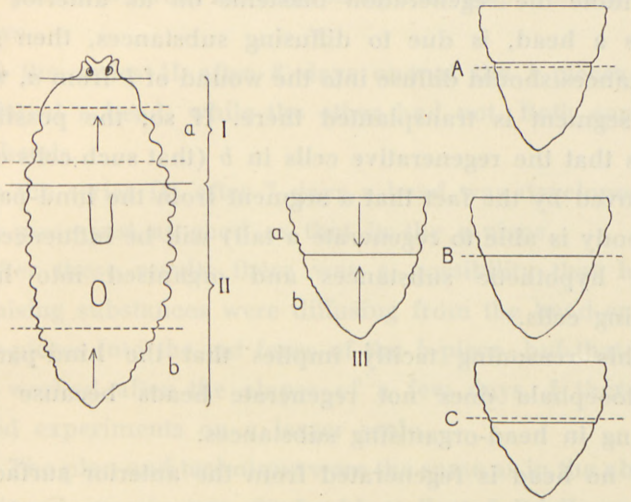


Fig. 13. *Bdellocephala punctata.* I, the part of the body which is able to regenerate heads. II, the part not able to do so. Dotted lines indicate cuts separating the segments *a* and *b*. Arrows indicate polarity. III, indicates the principle of the experiments: segment a is transplanted to the anterior surface of *b*, but with opposite polarity. A, B, C, the three ways of separating *a* from *b*, when these have been held together for some days. Dotted lines indicate cuts.

two parts have been in connection for some time and are then separated?

5. In Bdellocephala punctata I have found a planarian which possesses a head-frequency curve stopping a little before the pharynx. That is to say, only the fore-part of the body is able to regenerate a head. Moreover, this species proving very resistent to operations, my transplantation method could easily be employed on it.

6. The principle of the experiment is indicated in fig. 13. A piece *a* is cut out, transplanted autoplastically to the

piece *b*, but with reversed polarity (fig. 1, III). After a certain time it is again separated from *b*.

7. If the ability of the fore-part (when left to itself) to determine the regeneration blasteme on its anterior face to be a head, is due to diffusing substances, then such substances should diffuse into the wound of *b* from *a*, when this segment is transplanted there. If so, the possibility exists that the regenerative cells in *b* (that such cells exists is proved by the fact that a segment from the hind-part of the body is able to regenerate a tail) will be influenced by these hypothetic substances and organised into head-forming cells.

This reasoning tacitly implies that the hind-part of Bdellocephala does not regenerate heads because it is lacking in head-organising substances.

If no head is regenerated from the anterior surface of piece *b* after removal of the *a*-piece, this may be explained in different ways which will be discussed later.

8. A preliminary experiment was carried out as follows. 20 Bdellocephala which had been kept in the laboratory for about one month without food were handled as indicated above. Only 8 transplantations succeeded, and after 24 hours only 5 were left, the rest having cytolised. Two days after the transplantation the *a*-pieces were cut away as indicated in fig. 13c. I separated the pieces in such a way that the cut was laid in the *a*-piece but so as to leave only a brim of about $^1/_2$ mm. of the *a*-piece on the *b*-piece. By this procedure it is possible to prevent the cut face of the *b*-piece from contracting and closing up. If this takes place, no regeneration whatever occurs.

The separation was undertaken after the pieces had been held together for two days: 2 samples (series I); three days:

2 samples (series II); 4 days: 1 sample (series III). The cut-away *a*-pieces acted as controls.

In the series I after 9 days no head had appeared in the *b*-pieces, whereas the controls had developed heads after 6 days.

In the series II after 8 days one of the *b*-pieces had developed a head, while the other had not. Both controls had heads.

In the series III after 7 days a head was developed by the *b*-piece just as good as that in the *a*-piece.

After these results there was a possibility that head-organising substances were diffusing from the head-end of the *a*-pieces into the cut faces of the *b*-piece, but that they only worked after the elapse of a few days, I therefore started experiments on a larger scale.

9. The plan and technique were the same as in the above-mentioned experiments. 20 freshly collected Bdellocephala were operated on $^{21}/_{3}$-38. Ten of the grafts were successful. $^{22}/_{3}$, 20 Bdellocephala from the same stock were operated, 16 grafts were successful. These 26 grafts were allowed to adhere for 4 days. Then the *a*- and *b*-pieces were separated by cuts made in three fashions, grouped under A, B, C as seen in fig. 13.

In group A the cut was made in the *b*-piece, but as near as possible to the line of coalescense. In group B the cut was made in this line, and in group C it was made in the *a*-piece, but as near as possible to the said line.

The results that emerged were rather interesting.

In group A 8 experiments were made. 15 days after the separation of the *a*- and *b*-pieces all surviving specimens were fixed (Zenker with formol). At that time the control (pieces of other Bdellocephala from the fore-parts of the

body) had regenerated heads; this takes place after 6—8 days. None of the 8 *b*-pieces had regenerated traces of heads, several had a rather fine blasteme, but without any special stamp. Of the *a*-pieces 5 had died at this time. Of the three remaining specimens one had regenerated a fine head on its anterior surface, one had a blasteme, but no head, and

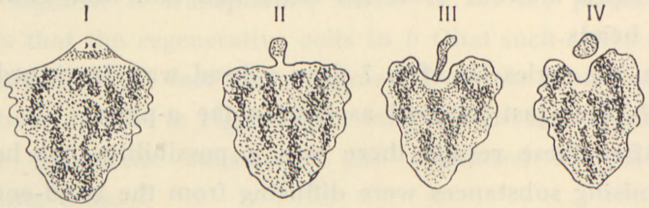


Fig. 14. *Bdellocephala.* 4 *b*-segments cut after modus fig. 13 C. I, a head is formed by the adhering *a*-brim. II—IV, various stages of a-brims being cast off from *b*.

one had no blasteme, but was rather cytolising at its anterior end.

In group B 11 experiments were made. The samples were likewise fixed after 15 days. Of the 11 *b*-pieces, none had heads, though several had beautiful blastemes. Only 5 *a*-pieces were alive. Of these 4 had made heads with eyes on their anterior surface.

Group C, 7 samples. After 15 days 6 *b*-pieces were alive. One had a beautiful head on its anterior surface (fig. 14 I), one had traces of eyes, 4 had no traces of head-building. 3 *a*-pieces were alive. One had a fine head on its anterior surface, one blasteme but without eyes, one cytolised on its anterior surface.

Only one or two *b*-pieces, in group C, had built a head. But on close examination it was quite clear that the head was made of and in the remaining tissues of the *a*-piece. This was also ascertained to be the case with the tail-pieces,

which had formed heads in the preliminary experiments. It will be remembered that in all these experiments the cut was made in the *a*-piece. That this is so can be clearly seen in fig. 14 I, where the light space between the *b*-piece and the head indicates the unpigmented tissue between host and graft.

After this it may be said with fair certainty that no agents from the *a*-pieces penetrate into the *b*-pieces, forcing these

Fig 15. *Bdellocephala*. *a*-segments. I, piece of *b*-brim in course of constriction. III, piece of *b*-brim only loosely adhering to *a*-segment, where *a* blasteme is formed. II, a new head is formed in the *a*-segment in accordance with the polarity, a piece of *b*-brim is pushed aside.

to organise heads. It is therefore in the highest degree improbable that dynamic polarity displays itself in the same manner as, say, the organising blastopore-lip, i. e. with organising substances.

10. Most of the pieces, after having been separated, had some tissue of the other piece adhering to them. This is of course the case in group A, where the *a*-pieces had some of the *b*-tissues fastened to them, because the cut was made in the *b*-piece. And so also—vice versa—in group C. In the B-group the line of coalescense was in most instances somewhat irregular so that the cut was not able to separate the two pieces cleanly.

The adherent tissue showed two distinct modes of behaviour. In some cases the strip of *a*-tissue covered the anterior surface of the *b*-pieces, and in one or two cases when *b*-tissue covered the surface of the *a*-wound, the

foreign tissue adhered so tightly and covered the wound so completely that no regeneration-blasteme was formed from the bigger piece.

It is very interesting to note that a very small brim of tissue from another part of the body is able to block the regenerating-powers of the piece on which it is grafted. This is shown in fig. 16, where a brim of tail tissue prevents the *a*-segment from forming head on its anterior surface.

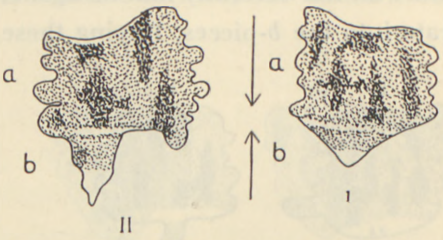


Fig. 16. *Bdellocephala*. I, an *a*-segment with *b*-brim, which covers the whole wound-surface. II, same, but *b*-brim only covering half of the wound-surface of *a*. Both *b*-brims are developing blastemes on their free (hind) surface, tending to become tail. The light spaces between *a* and *b* indicate unpigmented tissue on the border-line of the two segments.

But whereas the *b*-brim on an *a*-piece inhibit all regeneration from *a*, it is itself able, if big enough, to regenerate tail-shaped buildings (fig. 16 I, II). And an *a*-brim on a *b*-piece is itself able to build up a head, as we have seen. It should, however, be noted that this brim forms its head opposite to its own inherent polarity. That this phenomenon is certainly not due to directing forces from the *b*-piece is evident from the fact that very short pieces of planarians by Sivickis (1930) were shown to be able to develop heads on both anterior and posterior surfaces. It has by Morgan likewise long ago been demonstrated that very short head-pieces and tail-pieces sometimes regenerate their own doubles, "Janus-heads".

In other cases, the most numerous, the adherent strips gradually drew themselves together into rounded appendices

on the surface of the pieces to which they were grafted, eventually pushed aside by the growing blasteme (fig. 15 II). The connection between these lobes and the main-piece is gradually loosened, and in some of the samples the bases of the lobes were quite constricted and the lobes in a few cases had fallen off when the fixation took place. Several instances are shown in figs. 14 II—IV and 15 I—III.

This makes it clear that the remaining brim of tissue from the grafted but again separated piece is treated as foreign by the other piece, though it comes from the same animal, thus giving evidence of a chemical difference in the different parts of the body. But the experiments supply further evidence.

11. Why is the brim not always cast away? It is found that the brim-tissue is rolled off only when a blasteme is formed by the main piece. Now, is the blasteme formed because space is made for it by the active rolling off of the foreign tissue, or is the brim forced away by the blasteme? It has never been observed that the brim is fastened onto the blasteme. It is of course hard to say which is the effect and which is the cause. Perhaps the histological investigations will furnish the answer. But at the outset I should think that the formation of a blasteme is the primary cause of the pushing aside of the foreign tissue, for a clear line of demarcation is always seen between the blasteme and the lobes. And why should the brim not actively roll itself off when it covers the wound completely?

12. To serve as a control to these experiments the following were made. 10 Bdellocephala were decapitated on a level with the anterior surface of the *a*-pieces. Small blocks of sterilised 3 % agar (fig. 17 I) were laid firmly to the cut surface on Scotté-tables, the whole thing then

put into the cool room. The same day 5 Bdellocephala were transversally cut on a level with the anterior surface of the *b*-pieces (fig. 5 II). Agar-blocks were fixed to the wounds in just the same fashion as in the first experiment. This was to serve as a control for the following experiment.

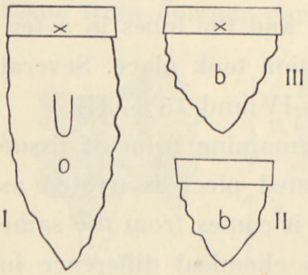


Fig. 17. *Bdellocephala*, I, agar-block firmly attached to wound made in the anterior part of the body. II, agar-block attached to a *b*-segment (tail), serving as a control to III: agar-block from I attached to wound of fresh *b*-segment to test whether hypothetical head-forming substances possibly taken up by the agar from I, are again given off in III to *b*-segments.

5 agar-blocks which had for 24 hours adhered to the cuts representing anterior surfaces of *a*-pieces, were now pressed against the wounds of 5 new *b*-pieces (fig. 17 III). If the agar-blocks had been imbued with head-forming substances, there would be a possibility that such substances would diffuse into the *b*-pieces and there act as organisers.

The agar-blocks were fixed to the *b*-pieces for two days and then removed. After 17 days the *b*-pieces were fixed. Neither these nor the 5 controls had formed heads in the blastemes.

Discussion.

The fact that the fore-part of the *b*-pieces does not react with head-formation in the blasteme which is built on the cut surface after the removal of the *a*-pieces may of course be explained in two essentially different ways.

1. The *b*-piece is unable either to suck up headforming

substances from *a*, or unable to respond to the summons of such substances after these have been sucked up.

2. The *b*-pieces do not react owing to the simple fact that no headforming substances are given off from the *a*-pieces.

We have to discern between these two possibilities.

My experiments on the possible organising capacities of the adult planarian head have shown that adult tissue does not react by reorganisation to possible influences from this head.

Only if a blasteme is formed is this in some way determined in accordance with the polarity or rather the possibilities (e. g. in the very short segments, where a head may be formed opposite to the natural polarity of the segment) of the adult pieces from which it is regenerated. Then the blasteme in its turn gives off forces which reorganise the adult tissues themselves.

Now, when piece *a* with reversed polarity is grafted into piece *b*, no formation of a blasteme takes place. Therefore no available tissue is able to respond to possible influences from either *a* or *b*. And the experiments show that the adult tissue does not react to eventually present summons from other adult tissue.

It therefore seems most probable that we have to look upon regeneration in planarians in different way from that usually adopted. It is commonly assumed that the various parts of the adult planarian body are able to organise, in fact it has been stressed (e. g. HUXLEY and DE BEER 1934) that the head of the planarian is equivalent to, say, the organiser of the amphibian embryo.

After the results of the transplantation experiments here presented, I think that the following working hypothesis may be formed.

Organising capacities in the adult planarian body are present only in a masked or latent manner. They can unfold themselves only when a blasteme is formed, and only in this blasteme, not in fully differentiated tissues. The blasteme is of course made of cells from the grown-up body itself, but only when the cells in the blasteme have been together for some unknown time are they made sensible to influences from the adult body, and then according to these influences determined in accordance with the polarity and capacities of said body. But then the blasteme takes the lead, and besides differentiating itself, gives off organising influences to the body, so strongly marked that they are able to remould the adult tissues (morphallaxis).

Therefore, only in the blasteme must we look for competent (Waddington) tissue, and for tissue containing organising forces comparable with the organisers in embryonic development. Adult tissue on the contrary is only competent when under the influence of forces generated in the blasteme.

So far, it has been impossible, with the new method, to transplant pure blastemes. I hope in the future to be able to develop a more refined technique, which should be able definitely to settle the question.

Further, and very strong, evidence that the formation of a blasteme is necessary for the rebuilding of the whole organism is afforded by the fact that when a wound is closed up, so that no blasteme is formed, no morphallaxis takes place. The forces necessary for morphallaxis therefore must be generated in the blasteme.

All this suggests that in the fully differentiated adult organism we have to deal with inhibiting factors controlling

the interaction of the various organs and cells. In the regenerating organism "organising" forces control the building up of the animal until a new level of interaction between the organs has been established.

If this idea be accepted, it is easily understood that the differentiated adult tissues do not exercise any organising influence on other adult tissue, as has now been shown for the head of *Planaria lugubris* and body-segments of *Bdellocephala punctata*.

Finally I should like to say a few words concerning the curious phenomenon of the rolling off of the tissue-brim grafted from another part of the body. When a blasteme is formed at the place of junction between the two grafted pieces, then the foreign tissues are pushed aside. Here again it seems that the blasteme has some qualities which are not present in the adult tissue.

Summary.

1. The head-frequency curve of Bdellocephala punctata is made out. Only the part of the body before the pharynx is able to regenerate a head.

2. The hind-part of the body is able to regenerate a tail with more energy than the fore-part; thus there also exists a tail-frequency curve.

3. Segments from the fore-part of the body of the planarian Bdellocephala punctata, which are able to regenerate heads on their anterior cut surface, are transplanted with the anterior surface to the anterior surface of tail-segments, which are not able to regenerate heads. This was done to test the question, whether headforming powers penetrate into the tail-pieces, forcing these to regenerate heads. The

segments were cut from the tails after they had been attached to them for a few days. The tails never regenerated heads.

4. If strips of the segments adhere to the tail after the removal, the strips may, if big enough, regenerate heads with a polarity in a reversed direction to the polarity of the segment itself.

5. Small strips either of segment or tail, or strips of tail-tissue on segments may inhibit further regeneration, if they cover the whole cut surface.

6. If the wounds on the anterior surface of either tail or segment are not closely covered by the strips of the other piece, a blasteme is formed, and then the strips are pushed aside and lastly cast off, being then treated as foreign tissue.

7. These results are discussed and their value for a working hypothesis concerning regeneration pointed out.

(Experimental-zoologiska avdelningen, Stockholms Högskola and Carlsbergfondets biologiske Institut).

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