Studies on Regulation.

VIII. Functional Regulation and Regeneration in Cestoplana.

By

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With 46 figures in text.

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Introduction.

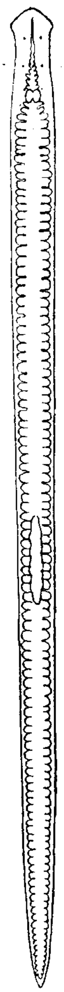
During my stay at the Zoological Station in 1902/3 a small polyclad which occurs in considerable numbers in fine sand — often together with *Amphioxus* —, was discovered to be excellent material for the study of certain regulative processes. Professor LO BIANCO informed me that the species was as yet undescribed but that he regarded it as a species of *Cestoplana*, hence I have used this name in the title.

The animal is from five to eighteen millimeters in length, extremely slender, in fact almost thread-like in appearance to the naked eye and is white in color by reflected light. Seen through the microscope the intestine is very dark, almost black, in color and opaque, the other regions of the body being semi-transparent. Fig. 1 represents a specimen somewhat above the average in size.

The cephalic ganglia are situated some distance from the anterior end of the head, a number of eyes, commonly six but with frequent variations are present on the dorsal surface anterior to the ganglia. The anterior end of the head is slightly broadened, between this region and the ganglia is a somewhat narrower region, from the ganglia to the pharynx which lies near the middle of the body, the width is nearly uniform, and finally, posterior to the pharynx the body usually tapers slightly to the tail.

My work necessitated numerous and careful measurements. Like other Turbellaria this is a difficult form to measure, but by the use of an ocular micrometer with a low power of the compound microscope I succeeded in obtaining very satisfactory results. The measurements were usually repeated one or more times as check, but after some practise the differences were not sufficient to affect the results appreciably. The measurements usually made are as follows: length of whole body, length of preganglionic region, length of prepharyngeal region, length of pharynx, width of the narrow preganglionic region, width at the level of the ganglia, width about 1 mm. posterior to the pharynx. When necessary additional measurements were made; for instance in some cases the width at the ganglia was not quite as great as at a level one millimeter posterior to them; in such cases each region was measured. From these measurements figures were plotted in my notes wherever special features made it necessary and from the measurements and the drawings in my notes the figures of the present paper are made.

Fig. 1.



The animals were found to be extremely hardy except after violent stimulation when they secreted a large amount of slime, remained contracted, with but little movement and usually died after a few hours. The operation itself frequently brought about the death of specimens or pieces in this manner, especially if the cut was not almost instantaneous or if adjacent parts were crushed by the turning of the lancet.

But if the pieces withstood the operation they could be kept alive for months even in Stender dishes with changes of water about every day ten days. Some pieces lived for nearly five months without food and were still alive when it became necessary to conclude my experiments.

The present paper is limited to the consideration of regeneration in the stricter sense, i. e. the formation of new tissue on cut surfaces. The consideration of the changes in proportion and of certain intestinal changes which are of great interest will follow. This use of the term suggested by Driesch is, I think, preferable to the general vague content given it by some writers. Careful definition and limitation of terms is of prime importance for correct analysis of these complex phenomena. It seems preferable for the sake of clearness and simplicity to consider different series of regulative phenomena under different heads rather than to describe from beginning to end the history of each piece considered, and then select the points of importance. This arrangement, while analytical rather than descriptive, affords as complete a survey of the facts as the other. As I have pointed out in previous papers (CHILD '04a, '04b, '04c), the behavior of the specimens is an important factor in regulation: a brief account of the behavior and motor activities precedes the account of the regulative processes.

As in the previous studies of this series the figures are somewhat diagrammatic. The intestine is indicated only in Fig. 1 and in cases where intestinal regeneration is concerned. Figs. 1, 19 and 37 are magnified about 7 diameters, the other figures about 11.5 diameters.

Behavior.

Unlike many of the polyclads, this form has never been seen to swim, though frequently individuals which happen to come into contact with the surface film leave the glass and creep beneath the film, apparently preferring it to the surface of the glass.

In creeping the body is extended to its full length and when movement is along a straight line the body is straight. Slight changes of direction to and fro which are frequently made often give the body a snake-like, simuous form, but this is not at all necessary for advance. The movements of the preganglionic region are extremely rapid and characteristic. They consist of lateral movements, dorso-ventral movements, and total or partial extensions and contractions. By means of these movements this part explores the whole region about the anterior end of the body as the animal advances. Contact with a moving object such as another individual causes instant contraction frequently not only of the head but of the whole body. Measurement of this region was possible only when the animal was moving over a smooth clean glass surface. The reaction to any slight irregularities was so intense that exact measurement was out of the question at such times.

From the ganglionic region posteriorly the wohle ventral surface is in contact with the substratum during locomotion. When the animal is moving rapidly it can usually be dislodged from the surface by a strong current suddenly directed against it from a pipette, but if any slight stimulus precedes the current dislodgement is difficult or impossible.

In all cases, however, the extreme posterior end is the chief organ of attachment. When the whole body contracts suddenly the posterior end remains fixed and other parts are drawn back toward it. When the attempt is made to dislodge the animals from the surface frequently all parts of the ventral surface except the posterior end will be torn away from the substratum and the animal will still retain its hold with this part alone.

Some slime is secreted in creeping, but the amount is greatly increased when the animals are violently irritated. Masses of rhabdites also appear in it at such times. It is probable that under ordinary conditions the attachment to the substratum over most of the ventral surface is merely adhesion in consequence of the presence of the slime. The animals react to various stimuli by applying the margins of the body more closely to the substratum, but whether special adhesive glands are present I do not know. At all events no part of the body can be attached so firmly as the posterior end. During progression the tip of the tail is always applied more or less closely to the substratum.

In consequence of these habits of movement the body is typically subjected to longitudinal tension as in the case of *Stenostoma* (CHILD '02) and *Leptoplana* (CHILD '04 a), and many other Turbellaria. In *Cestoplana* as in these other forms this longitudinal tension is a factor of great importance in regulation.

This form is in many respects exceptionally favorable for the observation of the effects of contraction upon the intestinal contents. Individuals like Fig. 1 where the intestine is filled are less favorable than those where the intestine is partly empty, e. g. specimens after two or three weeks without food. In such individuals it can be observed without difficulty that the effect of contraction of either end of the body is to force the intestinal contents toward the middle region. The terminal portions of the intestine being often compressed to such an extent that their lumen is nearly or quite obliterated. When general contraction occurs the contents of the intestine are forced from both ends toward the middle region and the intestinal branches in this region become more or less distended. BARDEEN ('01, '03) made observations similar to these upon *Planaria* and suggested in the earlier paper that the position of the pharynx is just posterior to the region of »greatest intestinal pressure«. In the second paper the same conclusion is stated but in somewhat different form, viz. that the pharynx is formed in or just posterior to the region toward which the intestinal contents are forced by general contraction.

My observations on *Cestoplana* indicate that the position of the pharynx is similar here. There can be no doubt that the pharynx in the normal animal is at least very near the region of equilibrium between anterior and posterior intestinal pressure. In other words the pharynx lies in or near the region which is functionally the middle of the body. The question as to whether the pressure itself has anything to do with the origin and growth of the pharynx is an entirely different matter and need not be discussed until after the experiments have been described.

The animals are negatively phototactic in ordinary light intensities. It was interesting to note that this reaction became less marked the longer the animals were kept in the laboratory where they were exposed to diffuse daylight.

A strong positive thigmotactic reaction was also observed. This likewise became less intense after the specimen had been kept for some weeks in clear water on smooth glass surfaces.

The behavior of pieces after the removal of the cephalic ganglia differs to a considerable extent according to the level at which the cut is made. After removal of the ganglia by a cut just posterior to them the motor activities do not differ widely from those of the normal animal, though all movements are less rapid and powerful. These pieces are capable of holding to the substratum by the posterior end almost as firmly as the normal individuals, they are able to extend to the full length, and the reactions approach those of the normal animals.

If, however, the cut be made half a millimeter or more posterior to the ganglia the behavior is markedly different. Movement apparently »spontaneous« does occur but attachment to the substratum takes places only when the piece happens to attain the proper position — frequently it moves along on the side — and in all cases attachment is less close than in the normal animal. Coordination is clearly more imperfect than in the pieces mentioned above and the characteristic reactions are less distinct and these pieces remain quiet for much longer periods than the others. Extension to the full length rarely occurs.

As the level of the cut becomes more and more distant from the anterior end the motor activities become less and less coordinated, until in cases where section is made posterior to the pharynx the piece is simply carried through the water by its cilia, striking against any obstacle that may lie in its path and unless its direction is altered by chance, pushing against it until fatigue ensues. When placed in water on a slide these pieces often strike the edge of the drop and if not watched will frequently keep pushing against the surface film until evaporation kills them. The power of extension is slight and such pieces usually grow relatively shorter as time goes on.

The difference in behavior between pieces cut posterior to the pharynx and those cut just posterior to the ganglia is much more marked than in *Leptoplana* (CHILD '04 a, '04 b). This point is of importance, for a corresponding difference in regulative power will be found to exist.

Regeneration.

Regeneration is usually a comparatively inconspicuous feature of the process of regulation in *Cestoplana*: the amount of new tissue which arises from a cut surface never reproduces more than a very small portion of the part removed except in the case of anterior regeneration anterior to the cephalic ganglia, where the part removed is completely regenerated.

Notwithstanding the small amount of regeneration my observations and experiments along this line have brought to light a number of interesting and important points some of which are valuable as data for comparison with the results of experiments on other forms. In this form as in *Leptoplana* (CHILD '04 b, '05), a relation between the central nervous system and regeneration exists, but with certain important differences.

Before turning to the experiments it is necessary to point out the difficulty in distinguishing the boundary line between the new and old tissues. The animal possesses no pigment in the body-wall and the only difference between the new tissue and the old is the greater translucency of the new parts. In many cases where the amount of regeneration is slight the new part becomes indistinguishable within a week, but in some cases some difference between new and old tissues is visible after two or three weeks, though it is usually impossible to determine the exact boundary line between them. In all cases where this boundary could not be clearly distinguished its approximate position is indicated in the figures by a dotted line. In cases where its position could be clearly seen the line is unbroken.

1. Anterior Regeneration.

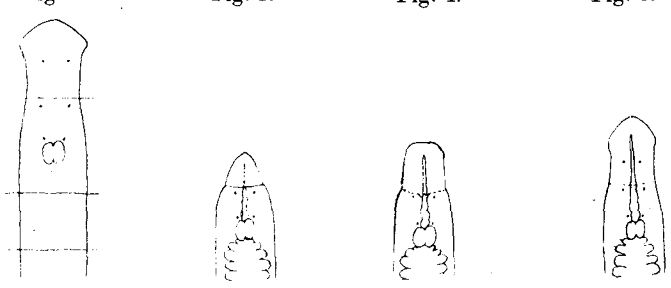
a. Anterior Regeneration in the Region of the Cephalic Ganglia.

In *Cestoplana*, as in *Leptoplana* (CHILD '05) all parts removed anterior to the cephalic ganglia are regenerated completely. But a marked difference exists between this form and *Leptoplana* as regards the relation between the cephalic ganglia and the regeneration of a head. In *Leptoplana* regeneration of the ganglia and head was not observed to occur after complete removal of the ganglia. In *Cestoplana*, however, regeneration of the ganglia and head is possible after section some distance behind the ganglia. In this respect *Cestoplana* resembles *Dendrocoelum* (LILLIE '01) except that the ability to regenerate the ganglia and head does not extend so far back in this case as that.

The following cases will serve as examples of the course and rapidity of regeneration at different levels near the cephalic ganglia.

Series 52. The results of section anterior to the ganglia are shown in Figs. 2—5. The level of section in this case was just anterior to the middle pair of eyes, as indicated by the transverse

Fig. 2. Fig. 3. Fig. 4. Fig. 5.



line anterior to the ganglia in Fig. 2. Fig. 3 indicates the condition six days after section, Fig. 4, twelve days, and Fig. 5, twenty-five days after section. It will be noted that the anterior end of the axial intestine has already extended into the new tissue at the stage of Fig. 3. At the stage of Fig. 5 regeneration is essentially complete:

the lateral lobes of the head are visible and the anterior pair of eyes has appeared in the new tissue. By this time it is very difficult to determine exactly the boundary between the new and the old tissue. Since neither is pigmented, the chief difference between them is in the somewhat greater translucency of the new tissue before differentiation is complete. Other similar cases did not differ essentially from this.

Unfortunately all my experiments upon the region anterior to the ganglia were made at approximately the same level as the case described. It is therefore impossible to determine whether as in *Leptoplana* (Child '04 c) the rapidity of regeneration increases with the size of the part removed. It is probable, however, that the results of section at different levels would be similar in both forms and in many others as well.

The removal of part of the preganglionic region is followed by increased motor activity of the head region. The searching movements and the tactile play of the parts remaining are more rapid and continuous than in the normal animal. Otherwise the behavior of these specimens is similar to that of uninjured specimens. The new tissue becomes functional within the first few days after its appearance: at the stage of Fig. 3 (six days after section) it is functioning in the characteristic manner as a tactile organ, though apparently rather imperfectly.

As will appear there can be little doubt that the extreme functional activity of this region is one of the factors that determine its rapid regeneration. No other part of the body shows so great regenerative power.

Section through the ganglia or immediately posterior to them is followed by regeneration of the ganglia and head, though the typical form of the head is developed rather slowly. Special description of these experiments is unnecessary since the results described below of other experiments at levels slightly posterior to this are essentially similar. After section at levels a short distance posterior to the ganglia the results differed in different individuals even when these were approximately equal in size. The following series afford illustration of this fact.

Series 51. Five specimens of approximately equal size with heads removed by section 0.3 mm. posterior to the ganglia (see the first line posterior to the ganglia in Fig. 2) constituted this series.

It is probable that slight differences in the level of the cut in different specimens existed, but examination of the pieces after section did not show marked differences in any case. For convenience the pieces are designated *A*, *B*, *C*, *D*, *E*.

Six days after section two pieces, *A* and *B* were like Fig. 6 and three, *C*, *D*, *E*, like Fig. 7, i. e. the amount of regeneration was greater in *A* and *B* than in the others.

During this period all the pieces were able to extend to the full length and to creep about in the dish, even when left undisturbed for hours or days, but their movements were considerably slower than those of the uninjured animal, the adhesion to the substratum was less close and the searching movements of the anterior end were slight. The pieces *A* and *B* seemed to be somewhat more active than the others.

Twelve days after section the pieces *A* and *B* were both about like Fig. 8. The new tissue had increased in amount and the axial

Fig. 6.

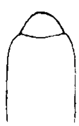


Fig. 7.

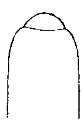


Fig. 8.

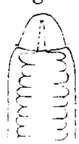
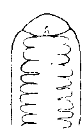


Fig. 9.



intestine had penetrated it. Pieces *C*, *D* and *E*, on the other hand, were all like Fig. 9, no marked change beyond a very slight increase in the amount of new tissue having occurred (cf. Fig. 7).

At this time the difference in behavior between the pieces *A* and *B* on the one hand and *C*, *D*, and *E*, on the other was much more marked than in earlier stages. All five of the pieces could creep slowly about as before, but the movements of *A* and *B* were more like those of the normal animal. It was possible to distinguish them from the others at once by their movements. The ability to coordinate was greater than in *C*, *D*, and *E*, and all movements were more rapid. One of the two pieces, *A* and *B*, seemed to show slightly greater coordinative power and rapidity of movement than the other. Hitherto these two pieces had been called *A* and *B* without distinguishing the one from the other. From this time on the more active piece was called *A*.

Twenty-five days after section the piece *A* was like Fig. 10. The new tissue was gradually assuming the form of a head and small new ganglia were visible. The piece *B* (Fig. 11) differed considerably from *A*. The increase in the amount of new tissue during the last thirteen days had been only slight and the outgrowth was still merely a mass with rounded margins. In both of these pieces the boundary between the old and new tissue was now very indistinct.

The pieces *C*, *D*, and *E* (Fig. 12), were all much alike. No marked changes had occurred during the interval except a slight increase in the concavity, i. e. a continued contraction of the old cut surface.

At this stage the behavior of the piece *A* was almost like that of the normal animal. The new parts performed the characteristic searching movements but were not yet fully functional: occasionally the piece endeavored to push out at the edge of the drop of water — a positive thigmotactic reaction, but one which the uninjured specimen does not show, it being apparently able to distinguish the surface film from solid surfaces.

Fig. 10.

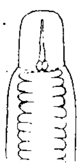


Fig. 11.

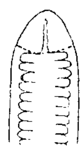
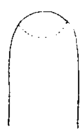


Fig. 12.



The motor activity of the piece *B* differed considerably from that of *A*. All movements were less rapid and the searching movements of the anterior end were only slight and seemed to be made with difficulty.

The pieces *C*, *D*, and *E*, behaved like pieces cut farther posteriorly the anterior end showing none of the characteristic activities of a head region. In fact these pieces were less active than during the earlier part of the experiment, while in *A* and *B* the activity had increased.

When the pieces were next examined (thirty-seven days after section) it was found that *A* and *B* were apparently infected; the new tissue was much contracted and no longer exhibited the characteristic motor activity. This condition followed an unusually long interval without change of water and similar changes appeared in the new tissue of other specimens subjected to the same conditions. No further regeneration occurred and the new tissue gradually contracted and became more opaque. After this change the pieces *A* and *B* were no more active than *C*, *D*, and *E*. Three weeks later (fifty-eight days after section, when the experiment was concluded) the pieces were all alive: *A* and *B* could be distinguished from the others by small irregular masses of tissue at the anterior end apparently undergoing disintegration, but the motor activity of all was about alike.

Series 50. This series consisted of three specimens of equal size with heads removed by section about 1 mm. posterior to the cephalic ganglia (see the second line posterior to the ganglia, Fig. 2).

Six days after section one of the pieces *A* was like Fig. 13, the other two (*B* and *C*) like Fig. 14. The amount of regeneration in *A* was much greater than in *B* and *C* and the axial intestine had penetrated the new tissue. A corresponding difference in activity could be observed, *A* being much more active and like an uninjured animal in its behavior than *B* and *C*. The new tissue in *A* was beginning to perform the characteristic movements of the head, though no traces of ganglia were visible as yet.

Fig. 13.

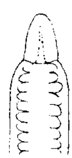


Fig. 14.

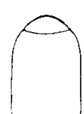
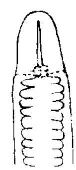


Fig. 15.



Twelve days after section *A* appeared like Fig. 15 while *B* and *C* showed no marked changes from the condition indicated in Fig. 14. In *A* small cephalic ganglia were now faintly visible. At this time the new head of *A* was performing the characteristic searching movements in a nearly normal manner and the general behavior of the piece differed little from that of the uninjured animal.

Twenty-five days after section *A* appeared like Fig. 16. Regeneration of the head was almost completed, and the behavior was practically normal. The condition of *B* and *C* is indicated by Fig. 17. Their behavior is like that of all pieces cut further posteriorly. Fifty-eight days after section the further changes in *A* were not great (Fig. 18). Two eye-spots were visible on the left side of the head. The motor activity was practically that of the normal animal. *B* and *C* remained in the same condition as before, as regards both form and behavior.

Certain features of these experiments require brief comment. Perhaps the fact of greatest interest in view of the previous similar experiments on *Leptoplana* (Child '05) is the occurrence of regeneration of the ganglia and head after complete removal of the ganglia and frequently from levels posterior to the ganglia. In this respect *Cestoplana* differs markedly from *Leptoplana*; in that species regeneration of the ganglia and head was not observed in any case after the ganglia were completely removed.

Another fact worthy of note is that in no case where the plane of section was an appreciable distance behind the ganglia was there any trace of regeneration of the region between the ganglia and the level of section. In all cases and at all stages (Figs. 10, 15, 16, 18) the regenerated ganglia were situated immediately anterior to the cut surface. Various cases of this kind have been described, e. g. *Planaria maculata* and most Oligochaetes which have been examined. This point will be discussed more fully in another connection.

Fig. 16.

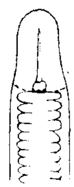


Fig. 17.

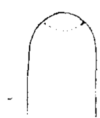
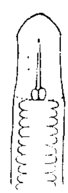


Fig. 18.



The results in the last two series (51 and 50) indicate very clearly that different individuals differ as regards regenerative power in a given region. Doubtless in Series 51 where five specimens were used there were slight differences in the level of the section in different cases, but in preparing the series all cases in which the cut was visibly more anterior or posterior were discarded. I do not think it possible that the level of the cut in any of the pieces of Series 51 could have been so far from the ganglia as that in Series 50 (see Fig. 2 the two lines behind the ganglia). Yet four pieces of Series 51 failed to regenerate ganglia and head. There seems to be no escape from the conclusion that regeneration of the ganglia and head is possible farther posteriorly in some individuals than in others. Attention has been called to the probable existence of a similar difference in different individuals of *Leptoplana* (Child '05), though in that form the region within which these differences usually occur does not extend so far from the ganglia as in *Cestoplana* — a difference perhaps to be expected since the latter form is greatly elongated.

Examination of the figures will show that in those pieces where regeneration of the head occurred the axial intestine penetrated the new tissue at an early stage (Figs. 3, 8, 13) while in cases where the head failed to regenerate the axial intestine regenerated very slightly or not at all (Figs. 7, 9, 12, 14, 17). It is probable that this difference is one of the results of the differences in motor activity. In the more active pieces the intestinal contents are forced into the peripheral parts of the intestine more frequently and with greater pressure. In *Leptoplana* the intestinal branches never enter the new tissue, at the anterior end to any extent except when ganglia are present and the head is regenerated, and the growth of the intestinal branches into tissue which is regenerating posteriorly differs widely according as the ganglia are present or absent (CHILD '04 b, and '05). These differences in *Leptoplana* were interpreted as primarily the result of differences in the intestinal pressure and the present case is undoubtedly similar.

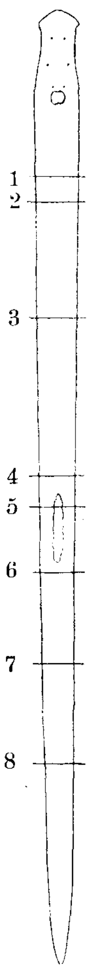
In the various cases described there is the same correlation between the power of reaction and coordination on the one hand and the amount of regeneration on the other. The pieces that are capable of regenerating the ganglia and head can be distinguished from the others by the difference in behavior. The power to regenerate the ganglia and head extends only a short distance posterior to the original ganglia and apparently differs in different individuals. It is evident however, that the anterior ends of the longitudinal cords in *Cestoplana* possess functional relations which in *Leptoplana* are confined to the cephalic ganglia. It is unnecessary to repeat what has been said in previous papers (CHILD '04 b, '05) regarding functional conditions as factors in regeneration. What has been said regarding *Leptoplana* apparently holds good for *Cestoplana*. Indeed the results in this case confirm and extend the conclusions reached from previous experiments.

b. Anterior Regeneration at Other Levels Posterior to the Cephalic Ganglia.

At all levels except near the cephalic ganglia the amount of anterior regeneration is small. In general, it may be said that it is somewhat greater anterior to the pharynx than posterior to it. In every case where a single individual was cut into several pieces the amount of anterior regeneration decreased with increasing distance of the cut surface from the anterior end. When different individuals are compared, however, it is found that some show a greater regenerative power at all levels than others, so that the amount of regeneration at a given level in the one may be greater than that at a more anterior level in the other.

This form is not particularly favorable for accurate conclusions along this line but a brief description of the results of my experiments will afford some points of interest. From among some fifty series several have been chosen for description: these were not selected with regard to the uniformity of results but simply with regard to the level at wich regeneration occurred. Since the individuals from which the pieces were obtained differed more or less in size I have found it convenient to reduce their measurements to a standard (average) size. Fig. 19 is drawn from these standard measurements and the levels of section in the different cases are indicated. The figures of the pieces, however, are not thus reduced to a standard. The different cases and the lines indicating the level of section in Fig. 19 are correspondingly numbered.

Fig. 19.



1. Figs. 20–22; four, eight and sixteen days after section: no further regeneration occurred after this time.

2. Figs. 23 and 24; five and ten days after section: no further regeneration.

3. Figs. 25 and 26; four and eight days after section: no further regeneration.

4. Figs. 27 and 28; four and eight days after section: no further regeneration.

5. Figs. 29 and 30; four and eight days after section: no further regeneration.

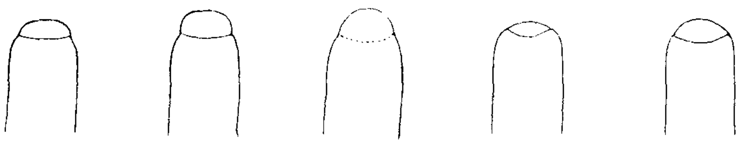
6. Figs. 31 and 32; five and ten days after section: no further regeneration.

7. Figs. 33 and 34; five and ten days after section: no further regeneration.

8. Figs. 35 and 36; five and ten days after section: no further regeneration.

The third figure (Fig. 22) in No. 1 and the second figure (Figs. 24, 26, 28, 30, 32, 34, 36) in each of the other cases indicates the maximum regeneration. If these are compared it is evident that with the exception of Nos. 5 (Fig. 30) and 6 (Fig. 32) the amount of regeneration decreases toward the posterior end of the body. The small amount of regeneration in No. 5 (Figs. 29 and 30) is probably due to the fact that in this case the cut was made through the pharynx: the contraction of the posterior portion of the pharynx has drawn the two sides of the cut together, leaving little of the cut surface exposed. The difference in regenerative power at different levels is slight but I think it is real. It will be noted that it is usually less marked in earlier than in later stages, i. e. in the more anterior pieces regeneration continues for a longer time than in the others. This is especially noticeable

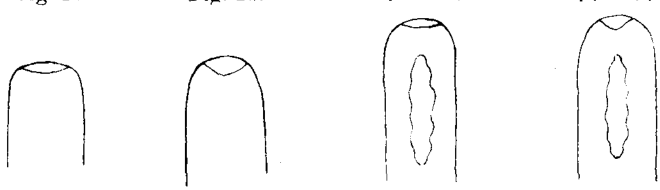
Fig. 20. Fig. 21. Fig. 22 Fig. 23. Fig. 24.



in No. 1 (Figs. 20—22) where growth of the new tissue continued for about a week longer than in the other cases.

Nos. 2 (Figs. 23 and 24), 6 (Figs. 31 and 32), and 7 (Figs. 33 and 34) of this series are pieces from a single individual, sectioned at the levels indicated in Fig. 19 by the numbers 2, 6 and 7. In these three pieces the difference in the amount of regeneration with difference in level is evident. The amount of regeneration in No. 2 (Fig. 24) is greater than in No. 6 (Fig. 32) and the same relation

Fig. 25. Fig. 26. Fig. 27. Fig. 28.



holds between this and No. 7 (Fig. 34). The data from single individuals are of course more convincing than those obtained by comparison of different individuals. In every case where a specimen was sectioned so that two or more anterior cut surfaces at different levels were present the results were similar to those obtained from pieces 2, 6, and 7, i. e. a decrease in the amount of regeneration with increasing distance from the anterior end was noted.

The cut surfaces in the pieces 1 and 2 (Fig. 19) are not far posterior to the level where complete regeneration of a head occurs (cf. Series 50 in the preceding section). It is of interest in this connection to note that in No. 1 regeneration is much greater in amount and continues for a much longer time than in any of the other pieces in this set.

As regards the activity and behavior of these pieces with anterior ends at different levels there is a marked difference. In all cases it is possible to distinguish by its behavior a piece with anterior end in the anterior third of the body from a piece cut posterior to the pharynx. The former is distinctly more active, is able to extend

Fig. 29.

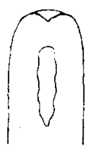


Fig. 30.

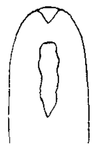


Fig. 31.

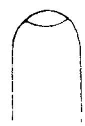
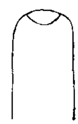


Fig. 32.



nearly to its full length, sometimes attaches itself and adheres by its posterior end, is able to right itself if turned over and if the anterior end be somewhere in the level of 1 (Fig. 19) it frequently shows some traces of the characteristic searching cephalic movements. These pieces frequently move slowly about in the dishes without apparent external stimulus.

A piece from the region posterior to the pharynx, on the other hand, behaves in a very different manner. Muscular activity is slight and consists chiefly of peristaltic contractions which pass

Fig. 33.

Fig. 34.

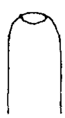


Fig. 35.

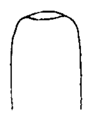
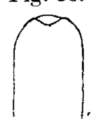


Fig. 36.



over the body from time to time, sometimes accompanied by slight extension, but in no case is the piece able to extend to its full length. The piece advances when stimulated but the cilia alone are used in progression and there is no marked reaction at the anterior end to tactile stimuli. The dorsal or ventral surface or either side may be uppermost during movement. After stimulation the activity gradually diminishes and the piece finally comes to rest, showing only occasional peristaltic contractions.

It is of course impossible to distinguish marked differences in the behavior of pieces with anterior ends at only slightly different levels. In such cases as the pieces Nos. 2, 6 and 7, however, which it will be remembered were taken from a single individual, differences in behavior could often be observed. The record for these three pieces in my notes is essentially as follows: piece *B* (No. 2), active: attaches itself to glass and creeps, though slowly, more often than either *C* (No. 6) or *D* (No. 7). Piece *C* (No. 6) moves about by actively means of cilia after stimulation, but in any position and does not attach itself to the substratum. Piece *D* (No. 7) swims in any position after stimulation but does not extend fully, and is in general less active than *C*. Other cases afford similar data.

It should be said that size of the pieces is not a factor in determining the result except in extreme cases. For example the piece No. 6 was less than half as long as piece No. 7, yet it was more active and shows a greater amount of regeneration than the other. On the other hand, pieces No. 2 and No. 7 were of almost equal size, yet differed widely as regards behavior.

We are, I think justified in concluding that the amount and complexity of motor activity of which a piece without ganglia is capable are determined to some extent by its position in the body. A similar conclusion was reached regarding *Leptoplana* (CHILD '05), though the differences were less marked in that form than here. If this conclusion is correct there can be little doubt that these differences in activity are primarily dependent upon the central nervous system. Admitting this, it follows that differences of some sort — very possibly differences in complexity of structural relation — must exist in different regions of the longitudinal nerve cords, from the ganglia posteriorly.

But the most interesting point is the parallelism between motor activity and regenerative power. In the case of pieces with anterior ends in the region between 1 (Fig. 19) and the cephalic ganglia it is often possible to determine by observation of the behavior during the first few days following section, whether they will regenerate a head or not. At other levels posterior to this region the differences both as regards activity and amount of regeneration are not so great and hence less easily distinguishable, though without doubt present.

c. Discussion of the Experiments on Anterior Regeneration.

It has been shown in earlier papers for both *Stenostoma* (CHILD '02, '03) and *Leptoplana* (CHILD '04 a, '04 b, '05) that regeneration and regulation in general may be determined in greater or less degree by the functional conditions affecting the part concerned. The conditions in the present case are similar to those in *Leptoplana*, and the same conclusion is justifiable, viz., that the relation between the nervous system and anterior regeneration is not direct but indirect, i. e., that regeneration is determined in some degree by certain functional conditions, these in turn being determined by that part of the central nervous system which is present in the piece. On this basis the parallelism between motor activity and regenerative power is explicable. The rapid regeneration of all parts anterior to the cephalic ganglia also receives its explanation. No other part of the body shows such intense motor activity.

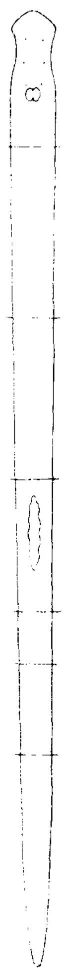
In comparison of the pieces from different levels it is found that regeneration of the ganglia and head is possible only when a certain degree of complexity and coordination of motor activity exists. Pieces falling slightly below this limit may show a much larger amount of regeneration than pieces which fall far below it, and some approach to head-formation may occur as in several cases described above.

The same possibilities that were discussed in the case of *Leptoplana* (Child '04 b, '05) are before us here viz. three: First: special formative stimuli may conceivably exist and may be related to the various parts of the nervous system in the same manner as functional stimuli: these may give rise to new parts. Second: the functional stimuli may exert a formative influence. Third: the functional conditions resulting from the effects direct and indirect of the functional stimuli upon the parts concerned may constitute formative factors. There is no good evidence in these cases in favor of the first possibility. As regards the second and third, we know that in many cases functional conditions not determined by the nervous system are important formative factors, hence the inference is justified that these conditions may constitute formative factors as well when they are as when they are not determined by the nervous system. For more extended discussion the reader is referred to the papers on *Leptoplana* (Child '04 a, '04 b, '05). The conclusions reached in these earlier papers are confirmed and extended by the results of my work on *Cestoplana*.

2. Posterior Regeneration.

In *Cestoplana* the amount of posterior regeneration is never great. During the first few days after section a slight outgrowth of new tissue is visible on all posterior cut surfaces posterior to the ganglia, but this has never been observed to continue more than ten days and usually ceases in a shorter time. The exact determination of the amount of new tissue formed presents certain difficulties: even in the earliest stages the visible differences between the new and the old are slight, the former being somewhat less opaque and rather more whitish in color. After about two weeks in the most favorable cases the new and old tissues cannot usually be distinguished, and since the dark intestine may enter the new parts before this time nothing remains to show that regeneration has taken place. Later stages afford no data regarding regeneration.

Fig. 37.



Except in certain cases to be described exact measurement of the regenerated parts is possible only during the first three or four days after section, because the boundary between old and new tissue is clearly visible only during this time. In the following figures this boundary is represented by an unbroken or a dotted line. The latter indicates that it was impossible to determine exactly where the old tissue ended and the new began.

The transverse lines 1—7 in the Fig. 37 indicate the levels of section selected for description, the various cases being correspondingly numbered: only the posterior ends of the pieces are shown in the figures. The different cases were obtained from worms of more or less different size, but for the purpose of indicating the level of section in Fig. 37 all are considered as reduced to the same size, as in the preceding section on anterior regeneration. The figures of the various pieces, however, are drawn from actual measurements without reduction.

A study of the different series of experiments recorded in my notes has led me to the conclusion that the level of section influences the amount of regeneration but little. In fact I am inclined to believe that all except very marked differences in the amount of regeneration are merely individual differences or the result of inaccurate measurements. As will appear, however, a real difference apparently exists in certain cases.

Qualitatively the new tissue is always the same; it merely forms the tip of the posterior end. It may be noted here in passing that the position of the new pharynx differs widely according to the level of section; this point will be considered more fully later.

The following cases are given as examples of posterior regeneration from various levels. The substitution of any other similar series for these would not alter the conclusions, for the differences are always slight.

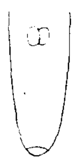
a. Posterior Regeneration in Pieces Containing the Cephalic Ganglia.

No. 1. Fig. 37, 1, indicates the level of section. Fig. 38 four days after section; Fig. 39, eight days after section: four days later the new tissue was not distinguishable from the old.

Fig. 38.

Fig. 39.

Fig. 40.



No. 2. Fig. 37, 2 indicates the level of section. Fig. 40, four days after section; Fig. 41, eight days after section; four days later new and old tissue were indistinguishable.

No. 3. Fig. 37, 3 indicates the level of section. Fig. 42, eight days after section: four days later new and old tissues not distinguishable. In this and in following series the earlier stages are not figured since they are similar to corresponding stages of Nos. 1 and 2.

Fig. 41.

Fig. 42.

Fig. 43.

No. 4. Fig. 37, 4 indicates the level of section. Fig. 43, eight days after section: new and old tissues almost indistinguishable.

In these four pieces, Nos. 1—4 the head and cephalic ganglia were present and uninjured and the pieces all resembled normal animals in behavior. Comparison of Figs. 39, 41, 42, 43, shows almost no difference in the amount of new tissue formed.

The results of all other similar experiments, about forty in number, were similar. No marked or constant difference in the amount of regeneration with difference of level could be discovered in pieces containing the cephalic ganglia.

Comparison of Figs. 39 and 43 shows how little the amount of regeneration is influenced by the size of the piece. Of these two pieces one is about five times as long as the other, yet the amount of regeneration is nearly the same in both.

b. Posterior Regeneration in Pieces without Cephalic Ganglia.

No. 5. In this case the head and cephalic ganglia were removed by section at the level 1 in Fig. 37. Fig. 37, 5 indicates the level of the posterior end, which is approximately the same as that of No. 3 described above. Fig. 44, fifteen days after section: new and old tissue nearly indistinguishable.

No. 6. This was a rather short piece without ganglia comprising that part of the body between the posterior end of the pharynx and 6 in Fig. 37. Fig. 45, ten days after section: the new tissue was still relatively distinct but five days later the boundary between new and old tissue had disappeared, though the tip was still slightly different in appearance from the rest.

Fig. 44.

Fig. 45.

Fig. 46.

No. 7. This piece was somewhat similar to No. 6, but longer, comprising that part of the body between the posterior end of the pharynx and 7, Fig. 37. Fig. 46, ten days after section: six days later new and old tissue were almost indistinguishable.

In these three pieces without the cephalic ganglia the differences in the amount of regeneration are not great. According to measurements and Figs. 44, 45, and 46 there is a slight decrease in the amount of regeneration toward the posterior end of the body. I think it probable, however, that this apparent difference is really more a difference in shape than amount. The width of the new tissue in Fig. 46 is greater than in Fig. 44 and 45, and the new part is shorter. Unfortunately my experiments include no other case without ganglia in which the level of the posterior end was so near the posterior end of the original specimen. But pieces without ganglia from other levels do not differ in any marked degree as regards the amount of posterior regeneration.

c. Discussion of the Experiments on Posterior Regeneration.

Perhaps the most striking results of these experiments are the small amount of regeneration in all cases and the absence of marked difference in the amount with difference of level. So far as my observations go it may be stated generally for this form that the amount of regeneration from a posterior cut surface is practically the same at all levels posterior to the cephalic ganglia, provided other conditions remain the same. Apparently functional conditions (CHILD '04 b) do not affect the amount of posterior regeneration in *Cestoplana.* Another result of interest is the apparently greater amount of regeneration in the pieces without ganglia, a result exactly opposite to that obtained in *Leptoplana* (CHILD '04 b). Moreover, the new tissue remains distinguishable from the old for a longer time in the pieces without ganglia than in those possessing the ganglia, as may be determined by referring to the description of the experiments.

It is evident that the conditions which determine posterior regeneration in this species must be widely different from those in *Leptoplana.* Moreover, the conditions which determine posterior regeneration differ from those which determine anterior regeneration in this form. As was noted in a preceding section anterior regeneration differs in amount to some extent with difference in level, while posterior regeneration does not. In the following section an attempt is made to determine the significance of these differences between anterior and posterior regeneration in *Cestoplana* and *Leptoplana.*

General Considerations.

Having described the phenomena of regeneration in *Cestoplana* it now remains to consider whether any satisfactory reasons can be discovered for the peculiarities which have been noted. The suggestions offered in the following paragraphs are the result of a comparative study of a number of different forms. Some of the experimental data are not yet ready for publication, but *Leptoplana* serves well for comparison with *Cestoplana* and the consideration will be confined chiefly to these two forms, for which the data are available. Further data on other forms which I hope to present later will serve to confirm the conclusions reached here.

It will be convenient to begin with the consideration of posterior regeneration. In the section on behavior it was noted that the posterior end is the chief organ of attachment, though any part of the ventral surface may be used in this way as in the case of *Stenostoma* (CHILD '02). After section of the body at any level posterior to the ganglia it can be observed that the anterior piece containing the ganglia uses the cut posterior end in much the same manner as the original tail. As will be shown later, this part elongates and becomes more slender in the course of a few days and, as regards its reactions and functional activity in general, does not differ perceptibly from the typical posterior end of the normal animal. In short a functional regulation has occurred in the course of these few days, in consequence of which the part which was brought by the operation into the position and relation to the other parts of a posterior end, has become functionally a posterior end. The change in functional activity is not very great in any case, but differs somewhat according to the level at which section was made.

The important point is that in this species the old tissues react by change in function and some slight changes in structure to the altered conditions. A region of the body which was not originally a posterior end becomes a posterior end in consequence of functional reaction to altered conditions.

Since this is the case the mechanical and other functional conditions resulting from the characteristic functional activity of this region, which were found to be of so great importance in determining posterior regeneration in *Leptoplana* (CHILD '04a, '04b) must affect the part which has altered its functional activity, i. e. they must be distributed over a larger or smaller portion of the old part. Of this part the cut surface with its regenerating tissue forms the terminal portion.

In *Leptoplana* the case is different. No marked degree of functional regulation occurs after removal of a posterior portion. *Leptoplana* uses the margins of the posterior half or two thirds of the body as organs of attachment and when the section is made anterior to this region the piece is unable to adhere to the substratum by its posterior end until the new tissue has appeared and become functional (see CHILD '04a, '04b). In other words, the posterior portion of a piece after removal of the original posterior end does not undergo functional regulation, its reactions do not change in any marked degree, and it does not represent functionally the part removed. Instead it retains the same functional activity and essentially the same structure and form as before section.

We may now turn to the consideration of the conditions affecting regeneration in the two forms. As I have endeavored to show (CHILD '04a), the removal of a part in such manner as to leave a cut surface alters conditions in the region about the cut surface in various respects. The mechanical equilibrium of the tissues is destroyed and, if the parts are plastic, cells or tissues may be forced out through the opening by the pressure upon them of other parts, which is no longer antagonized by pressure in the opposite direction. In addition to this the altered conditions usually bring about cell-division and growth which in most cases is doubtless a much more important factor. These processes constitute the initiation of regeneration and in forms where the cells are capable of reaction, they occur on all cut surfaces indifferently. In my previous papers on *Leptoplana* (CHILD '04a, '04b, '05) the experimental data favor the view that the further history of the new tissue appearing upon a cut surface is determined primarily by the functional conditions to which it is subjected, or in other words by its functional relations to the old part, which is dominant since it is fully organized and in functionally active condition. It was shown in these papers that both the amount of regeneration and the kind of structure resulting were dependent upon these functional conditions. In cases where functional activity was slight the process of regeneration ceased at a very early stage and in general the duration of regeneration and the amount of new tissue were clearly correlated with the degree of functional activity in the part concerned.

Considering for example the case of the regenerating posterior end in *Leptoplana* we find that as soon as the new tissue becomes functionally active at all it is subjected to conditions much like those to which the part removed was subjected, though usually more intense or »concentrated« since the new part is smaller than the old. In *Cestoplana* on the other hand, the old part adjoining the posterior cut surface has acquired the function of the part removed; it has become the tail. The new tissue at the cut surface appears in consequence of the initiatory stimuli, but the functional conditions which in *Leptoplana* bring about further growth and differentiation of the new part are in *Cestoplana* applied almost wholly to the old tissue anterior to this. I shall show elsewhere that this old tissue reacts to these conditions by elongation, which is probably due at least in part to actual growth. In short, the process of regeneration at the posterior end never passes beyond the initiatory stage in *Cestoplana* because no stimuli which can bring about further growth are applied specially to this region. Or the matter may be put thus: by the time the new tissue appears the parts anterior to it have been functionally transformed into a »tail« to such a degree that nothing remains for the new tissue to form except the extreme tip. In *Leptoplana*, on the other hand, where no such regulation occurs in the old tissue the regenerating part represents functionally, however incompletely, all or nearly all that was removed and so acquires the characteristic structure of the parts which it replaces.

I am inclined to believe that a similar relation between »redifferentiation« and regeneration will prove to exist in many cases.

If functional conditions influence regulative processes at all, and I think I have shown in previous papers that they do, it follows that the amount of actual regeneration (in the stricter sense) will vary inversely as the degree of regulation in the old tissues. The group of Turbellaria alone furnishes examples illustrating various phases of this relation. In *Stenostoma* for example (CHILD '02, '03) where the posterior end of a piece very soon represents functionally the parts removed, there is practically no regeneration from the cut surface. In *Cestoplana* functional regulation and consequent form-regulation of the old parts adjoining a posterior cut surface is almost complete and regeneration ceases at a very early stage. In *Planaria maculata* there is, as I shall show in another paper, some degree of functional regulation in the old tissue. Hence the process of regeneration is limited to forming the terminal regions of the body, and the amount of regeneration, both anterior and posterior, is about the same at all levels of the body (MORGAN '98, '00).

In *Planaria lugubris* (MORGAN '01a) on the other hand both anterior and posterior regeneration differ in amount according to the size of the part removed. While I have not had opportunity to examine this form it is probable that little or no functional regulation occurs in the old parts. And finally in *Leptoplana* (CHILD '04a, '04b, '05) there is practically no regulation in the old parts and the whole regulative process is regeneration.

The suggestion which has been made by several authors that the amount of regeneration in certain cases depends on the amount of undifferentiated or »embryonic« material present does not afford a satisfactory explanation of such differences as these. The attempt has been made (MORGAN '01 b) to account in this manner for the fact that *Planaria maculata* regenerates from the anterior cut surface only a head, no matter how far from the anterior end the level of the cut surface may be. It is difficult to reconcile the capacity of *Planaria* for repeated regeneration with a limitation in the amount of available material. Again a small piece of *Planaria* may regenerate many times the relative amount of material which a large piece produces, moreover, other cases will occur to all familiar with the facts where any explanation based on the amount of material available is unsatisfactory.

As we learn more of organic life and especially of the lower forms, it is becoming more and more evident that except in the last stages of starvation, »material« is available where the stimulus is sufficiently strong, or as we often say »where it is most needed«. The »material« for regeneration will usually be present when the stimulus is present. I have endeavored above to point out why the stimulus differs so widely in degree in different cases.

It is only natural to expect that this process of regulation in fully organized parts, or as we may call it, »redifferentiation« will occur more readily where the function of the part removed is relatively simple and does not differ very widely from that of the remaining part. It is probable that no functional part of an animal organism can be removed without bringing about in the remaining parts some degree, however slight, of functional regulation. In the more complex forms, however, this is commonly so slight that no great changes in structure occur, and the part is replaced, if replaced at all, by regeneration. But other features besides complexity of structure are concerned. *Leptoplana* is not, so far as we can judge, very different from *Planaria* as regards complexity, yet in the one case no great degree of regulation of old parts occurs, in the other such regulation is considerable. Differences of this kind may be due in part to differences in the degree of functional differentiation of different parts. If this difference is too great for regulation of the old parts to occur readily the conditions favor regeneration. It is probable also that the tissues of certain species react more readily than those of others to altered conditions: slight differences of physical or chemical composition may be responsible for such differences.

We must suppose that after removal of a part both functional regulation of the old part and regeneration begin. The result will depend on the relative rapidity of these two processes. If functional regulation occurs before regeneration has advanced far, as is the case in posterior regeneration in *Cestoplana*, then little regeneration will occur. If on the other hand functional regulation occurs slowly or not at all regeneration will proceed until the functional complex is essentially complete.

If we accept the views proposed above, it is evident at once why the amount of posterior regeneration in *Cestoplana* does not differ with the level of section, as it does in *Leptoplana*. Functional regulation is essentially complete at the posterior end of the piece in *Cestoplana* before the regenerative processes have gone beyond the earliest stages and there is no further stimulus to the localized growth of new tissue from the cut surface. As we shall see the growth is probably diffuse in the parts which have undergone functional regulation.

But the amount of posterior regeneration apparently does differ according as the cephalic ganglia are present or absent. The relation between the ganglia and the amount of regeneration is, however, exactly the opposite of that found in *Leptoplana* (Child '04 b). All of my experiments along this line indicate that the amount of posterior regeneration is greater when the ganglia are absent than when they are present. It would appear at first glance that this result must constitute a serious objection to the views advanced in the papers on *Leptoplana* (Child '04 a, '04 b, '05); since in *Cestoplana* as in *Leptoplana* the pieces without ganglia are less active than those in which the ganglia are present and the complex of functional conditions, which, in the papers above-mentioned, was regarded as an important factor in regeneration, is quantitatively reduced by removal of the ganglia.

But, as has been shown in the previous paragraphs, the functional conditions connected with motor activity can exert no appreciable influence upon posterior regeneration in *Cestoplana*, since functional regulation takes place in the parts anterior to the cut surface and there is no stimulus to continued growth of the regenerating parts. Evidently some factors other than the functional conditional must be responsible for these differences. One characteristic difference between the pieces with and those without ganglia is the difference in size of the cut surface. A comparison of Figs. 39, 41, 42, and 43 with Figs. 44, 45, and 46 will show at once that in the first set — pieces with ganglia — the cut surface at the posterior end is much reduced in size by the change in form of the old parts, while in the second set — pieces without ganglia — the change in form is much less. This difference in the rapidity of form-change is in agreement with the views expressed in previous papers (CHILD '02, '03, '04 a, '04 b, '05) according to which the mechanical conditions connected with the use of the posterior end as an organ of attachment are important factors in determining the form of this region, and the differences in size of the cut surface conditioned by it may exert some influence upon the amount of regeneration. If physical factors such as surface tension and capillarity play a role in determining the form of the new part it might be expected for instance that the curvature of the surface of the cell-mass growing out from the cut surface would be approximately the same so far as not altered by special mechanical conditions in individual cases. Examination of Figs. 39, 41, 42—46 shows that no very great differences in the outline of the new tissue appear. As has been pointed out above, functional conditions exert no appreciable influence on the amount of actual posterior regeneration and it is not at all improbable that since this is the case the physical relations of the cells may be important factors in determining the form of the new part. If this suggestion is correct the amount of regeneration will increase with the increase in size of the cut surface.

Attention was called above to the fact that the boundary line between the old parts and the regenerated tissue remains distinct for a longer time in pieces without than in pieces with ganglia. This difference is undoubtedly to be interpreted as a difference in the rapidity of differentiation in the new parts. In the piece with ganglia the characteristic functional activity of the posterior end, both new and old parts, is far greater than when the ganglia are absent; the posterior end of the body is attached much more frequently and more firmly to the substratum. It would be strange if the small new part did not acquire the characteristic structure and appearance in less time than in the pieces without ganglia. In these latter attachment is infrequent and slight and the functional activity of the new parts is much less. The difference in appearance between old and new tissue is never great and greater functional activity in the one case is quite sufficient, to my mind to account for the slightly more rapid change. It is of course impossible to determine how much regeneration occurs after the boundary between the old and the new tissue is obliterated. That a certain amount of growth does occur in the posterior portions of the pieces with ganglia appears probable from a comparison of the position of certain landmarks in different stages, a point which will be considered later. In any case this growth is not limited to the new tissue.

According to these suggestions the amount of posterior regeneration is determined simply by the local conditions at the cut surface and shows no relation to the size of the part removed. If there were no functional regulation and consequent redifferentiation of the parts anterior to the cut surface we should to expect to find the amount of posterior regeneration proportional to the size of the part removed, as in *Leptoplana* (CHILD '04 b).

On the other hand the rapidity of differentiation is determined by the functional conditions. In the pieces with ganglia in which the posterior end is used to a much greater extent differentiation is more rapid than in those without ganglia.

A comparison between posterior and anterior regeneration in *Cestoplana* also affords some important data regarding the relation between regeneration and redifferentiation. The amount of anterior regeneration, unlike that of posterior regeneration, varies considerably at different levels (see pp. 267—277), being greatest in the region of the cephalic ganglia and decreasing at first rapidly then slowly with increasing distance from the anterior end. In short, anterior regeneration differs widely in amount with differences of level, while posterior regeneration does not.

In this case, as in so many others, it is the functional activity of the parts in question that gives us the key to these differences. We have seen above that removal of a posterior part is followed by functional regulation in the old parts anterior to the cut surface, in consequence of which these parts take the place almost wholly of the part removed and there is no stimulus to regeneration except the initiatory stimulus resulting from altered conditions at the cut surface. If, on the other hand, a piece be removed from the anterior end no marked degree of functional regulation occurs. If, for example, the head is cut off immediately posterior to the cephalic ganglia the part remaining does not supply the place of the head, though its activity may increase somewhat. The new tissue arising from the cut surface is the only part which represents the head and functional conditions determine its development into this structure. In the case of *Leptoplana* the amount of new tissue regenerated was found to be more or less closely proportional to the size of the part removed, when the new part was the functional representative of the part removed. Anterior regeneration in the region of the ganglia in *Cestoplana* is essentially similar. Here only regeneration is qualitatively complete, and approaches quantitative completeness. The amount of anterior regeneration in this region is far greater than the amount of regeneration from any other region of the body, presumably because of the intense functional activity of the head-region and the absence of functional regulation in the old parts.

But at levels more than a very short distance posterior to the ganglia, the amount of regeneration not only decreases, but the regeneration of a typical head does not occur. Here evidently the conditions obtaining in the anterior region of the old part are not sufficiently like those in the head region even to start the new tissue along the line of head-development. The case of *Leptoplana* is similar.

The results of this comparison may be summed up as follows: when the functional activity of the part remaining after section is not too widely differentiated from that of the part removed it may react to the altered conditions and supply the place of this part, i. e., functional regulation and in consequence redifferentiation may occur. In such cases a complete individual results, but with little or no regeneration, e. g., *Cestoplana* after the removal of posterior parts, or *Stenostoma* after removal of any part (CHILD '02, '03). When, however, the part remaining after section is so different from the part removed that it cannot supply its place in the functional complex one of two results may occur: in case the part remaining resembles the part removed in some of its fundamental characteristics the new tissue growing out from the cut surface may regenerate the missing part more or less completely according to circumstances: e. g., regeneration of a head in the region of the cephalic ganglia in *Cestoplana* and *Leptoplana*[1]) (CHILD '05), and posterior and lateral regeneration in *Leptoplana* (CHILD '04 b, '05): in such cases the amount of regeneration will be more or less completely proportional to the size of the part removed. But, on the other hand, when the part remaining differs so widely from the part removed that none of the essential characteristics of the latter remain, regeneration of this part does not occur. In such cases

[1]) Attention has been called repeatedly both in this paper and in those on *Leptoplana* (CHILD '04b, '05) to the fact that regeneration of the head after total (*Cestoplana*) or partial (*Leptoplana*) removal of the ganglia took place only when the pieces showed some approach to the typical behavior of animals possessing the ganglia.

also the result differs according to circumstances: when the remaining part simply lacks definite functional characteristics no definite structure regenerates, e. g., anterior regeneration in *Leptoplana* (CHILD '05) and *Cestoplana* from levels distant from the cephalic ganglia. In these cases the greater the distance from the ganglia the less definite the functional activity and the less the advance in regeneration beyond the initiatory stage, as the facts show very clearly. In some cases, however, it may be that the part remaining does not show the characteristics of the part removed but those of another part: the result in such cases is so-called heteromorphosis. MORGAN ('97, '02) has shown that pieces cut from the posterior regions of the earthworm regenerate tails at both ends; in other words, pieces from this region retain none of the head forming factors and can react to the altered conditions only in the one way. Certain cases of heteromorphosis in *Planaria* probably also belong here, but further discussion is postponed until more facts can be considered.

Having now reached the point where the facts permit the consideration of this relation between functional regulation and form-regulation it will be necessary to refer to them frequently. It should perhaps be said that the evidence already presented in support of these conclusions constitutes only a part of that upon which they were originally based. But since the facts available for consideration are now sufficient to afford a basis for these views it has seemed advisable to present them here as a working hypothesis to which further data may be referred.

Summary.

1) Anterior regeneration in *Cestoplana* differs according to the level of section. Section anterior to the ganglia, through the ganglia, and immediately posterior to them is followed by complete regeneration. Anterior regeneration from levels posterior to these never gives rise to a new head, is much less in amount, and decreases with increasing distance from the anterior end.

2) In all cases where regeneration of a head is possible the behavior and reactions of the anterior part of the piece approach in some degree those of the normal animal. Where regeneration of the head does not occur this is not the case.

3) Posterior regeneration in *Cestoplana* is always very slight and does not differ appreciably in amount at different levels of the body.

4) In pieces without cephalic ganglia the amount of posterior regeneration is apparently somewhat greater than in pieces with ganglia. This difference is probably due primarily to difference in the size of the cut surface, which in the pieces without ganglia is greater than in those with ganglia. And finally, the difference in size of the cut surface is the result of the difference in activity. In the pieces without ganglia the posterior region becomes elongated and more slender in consequence of the characteristic functional activity, while in the pieces without ganglia the change in form of this region is less marked.

5) The new tissue formed at the posterior end becomes indistinguishable from the old parts earlier in pieces containing cephalic ganglia than in those without ganglia. This difference also is probably due to the difference in functional activity of this part in the two cases.

6) The small amount of posterior regeneration in *Cestoplana* is due to the fact that the old parts anterior to the cut surface supply almost completely the place of the part removed, i. e., they undergo functional regulation. Hence there is no stimulus to regeneration at the posterior end beyond the initiatory stages. At the anterior end however, no appreciable functional regulation of the old parts occurs, therefore regeneration of the missing parts occurs, provided the old part retains a sufficient degree of the characteristic functional activity to serve as a functional stimulus to regeneration.

7) Comparison of this form with *Leptoplana* and *Stenostoma* leads to the conclusion that redifferentiation of old parts is the result of a regulative functional reaction to the altered conditions after section; in cases where this functional regulation is complete or nearly so, regeneration in the stricter sense does not occur. In cases where it is incomplete or does not occur regeneration is possible, the amount of regeneration varying inversely with the degree of functional regulation and depending also on other conditions. In case the functional conditions in the region of the cut surface of the old part differ widely from those characteristic of the part removed, something different from that part may be regenerated: if this difference in conditions is simply absence of special functional activities the regenerated tissue may form no definite part (failure to regenerate a head in regions posterior to the cephalic ganglia); if on the other hand for any reason conditions characteristic of a part of the body different from that removed exist in the piece then the new

tissue may form this other part instead of the part removed (heteromorphosis).

Hull Zoological Laboratory, University of Chicago, July, 1904.

Zusammenfassung.

1) Die vordere Regeneration von *Cestoplana* weist je nach dem Niveau des Schnittes Verschiedenheiten auf. Auf einen Schnitt vor den Ganglien, durch die Ganglien, oder unmittelbar hinter ihnen folgt völlige Regeneration. Die Regeneration nach vorn von weiter nach hinten gelegenen Niveaux aus läßt niemals einen neuen Kopf entstehen, zeigt einen weit geringeren Betrag und nimmt mit wachsendem Abstand vom Vorderende ab.

2) In allen Fällen, in denen Regeneration eines Kopfes möglich ist, stimmt das Verhalten und die Reaktionen des Vorderendes des Stückes bis zu einem gewissen Grade mit denen des normalen Tieres überein. Wo keine Regeneration des Kopfes eintritt, ist dies nicht der Fall.

3) Die hintere Regeneration ist bei *Cestoplana* stets sehr unbedeutend und zeigt keinen nennenswerten Unterschied in ihrem Betrage in verschiedenen Körperhöhen.

4) In Stücken ohne Kopfganglien ist der Betrag der hinteren Regeneration anscheinend etwas größer als in Stücken mit Ganglien. Dieser Unterschied beruht wahrscheinlich primär auf dem Größenunterschiede der Schnittfläche, die in ganglienlosen Stücken größer ist als in ganglienhaltigen. Und schließlich ist der Größenunterschied der Schnittfläche das Ergebnis eines Aktivitätsunterschiedes. In den ganglienhaltigen Stücken wird der hintere Bezirk länger und schmäler infolge der charakteristischen funktionellen Aktivität, während in den ganglienlosen Stücken die Formänderung dieses Bezirkes weniger ausgesprochen ist.

5) Das neugebildete Gewebe am Hinterende wird in Stücken mit Kopfganglien eher ununterscheidbar von den alten Teilen als in ganglienlosen. Auch dieser Unterschied beruht wahrscheinlich auf der Verschiedenheit der funktionellen Aktivität dieses Teils in den beiden Fällen.

6) Der geringe Betrag der Regeneration nach hinten bei *Cestoplana* beruht auf dem Umstande, daß die alten Teile vor der Schnittfläche so gut wie ganz den verlorenen Teil ersetzen, d. h. sie unterliegen einer funktionellen Regulation. Daher besteht kein Reiz zur Regeneration am Hinterende über die Anfangsstadien hinaus. Am Vorderende jedoch findet keine nennenswerte funktionelle Regulation der alten Teile statt, daher tritt Regeneration der verlorenen Teile ein, vorausgesetzt, daß der alte Teil einen genügenden Grad der charakteristischen funktionellen Aktivität behält, um einen funktionellen Reiz für die Regeneration zu bilden.

7) Vergleich der vorliegenden Form mit *Leptoplana* und *Stenostoma* führt zu dem Schlusse, daß Wiederdifferenzierung alter Teile das Ergebnis einer regulatorischen funktionellen Reaktion auf die veränderten Verhältnisse nach dem Schnitt ist; in Fällen, wo diese funktionelle Regulation vollständig oder fast vollständig ist, kommt es nicht zur Regeneration im eigentlichen Sinne. In Fällen, wo sie unvollständig ist oder gar nicht eintritt, ist Regeneration möglich, wobei ihr Betrag im umgekehrten Verhältnis zum Grade der funktio-

nellen Regulation schwankt und auch von andern Verhältnissen abhängig ist. Unterscheiden sich die funktionellen Verhältnisse in der Gegend der Schnittfläche des alten Teils erheblich von den für den verlorenen Teil charakteristischen, so kann etwas von ihm Verschiedenes regeneriert werden: Wenn dieser Unterschied der Verhältnisse lediglich im Fehlen spezieller funktioneller Aktivitäten besteht, so kann das regenerierte Gewebe einen speziell differenzierten Teil nicht bilden (Mangel der Kopfregeneration in Bezirken hinter den Kopfganglien); wenn anderseits aus irgendeiner Ursache für einen andern als den verlorenen Körperteil charakteristische Verhältnisse in dem Stück vorhanden sind, dann kann das neue Gewebe diesen andern Teil bilden an Stelle des entfernten (Heteromorphosis).

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