FORM–REGULATION IN CŒLENTERA AND TURBELLARIA

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The following is a brief report of the more important results obtained during the author's occupancy of the table of the Smithsonian Institution in the Zoölogical Station at Naples, from July to December, 1903.

Being convinced by study of various fresh-water Turbellaria, and notably of *Stenostoma*,[1] of the possibility of experimental analysis and control of various factors of form-regulation, my chief purpose in visiting Naples was to obtain further evidence along this line. The forms employed for most of the work were the polyclad turbellaria, *Leptoplana tremellaris* and a small species of *Cestoplana*, which, so far as I could determine, was undescribed, and several species of the sea-anemone, *Cerianthus*, especially *C. solitarius*, this species being the most abundant and best suited in other respects for experimental work. In addition an experimental study of regulation in *Tubularia* was made, primarily for the purpose of reëxamining the remarkable regulative phenomena described by other authors. In this work several new results of some importance were obtained.

I. LEPTOPLANA

This form, one of the more common polyclads about Naples, was already known to possess a high degree of regenerative power. In one respect the relation of regeneration to the nervous system was conspicuous; in no case was a piece able to regenerate a head or cephalic ganglia. All portions of the head removed by cuts anterior to the ganglia or through their anterior portion were rapidly regenerated; if, however, the cut passed just posterior to the ganglia, the anterior regeneration was very slight—only sufficient to heal the wound—and a new head never appeared. Regeneration in the posterior direction was not, however, directly influenced by the presence or absence of the cephalic ganglia. Pieces from the anterior parts of the body, but without cephalic ganglia, regenerated at their posterior ends pharynx, genital organs, intestine, and all posterior parts

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[1] Cf. Studies on Regulation, *Roux's Archiv*, 1902–'03.

with the same rapidity as pieces containing the ganglia. It is evident therefore, that there is no direct relation between the presence of the cephalic ganglia and regeneration in general, the presence of the ganglia being necessary merely for the regeneration of the head.

During the course of regeneration the form of both the new tissue and the old parts becomes more or less altered. In specimens without food there is of course a reduction in size, but in addition to this the pieces become relatively longer and more slender (" morphallaxis," Morgan). An extended study of these phenomena was made and distinct confirmation of the conclusions reached in regard to *Stenostoma* was obtained, viz., that this change in form is due primarily to the tension to which the tissues are subjected by the movements of the animal, and especially by the antagonism between attachment to the substratum by the posterior end and the force of the forward movement in consequence of muscular or ciliary activity. The rapidity of this change in proportion depends on the degree of activity of the individual, the rapidity and frequency with which it moves about, and consequently the longitudinal tension to which its tissues are subjected. One case serves as an illustration: Pieces deprived of the cephalic ganglia are incapable of carrying out the typical creeping movements; they are able to advance very slowly after strong stimulation, but remain quiet most of the time when undisturbed. As was noted above, absence of the cephalic ganglia does not directly affect regeneration at the posterior end of a piece. With these two facts in mind, two sets of pieces were prepared, the posterior ends of all pieces representing the same level (anterior to the middle) of the body. From one set heads and cephalic ganglia were removed; the other set retained these organs uninjured. The pieces of the latter set moved about actively, those of the former showed scarcely any power of movement. In the latter set the bodies, and especially the plastic regenerated tissue, gradually assumed a tapering form with greatest breadth at the anterior end and pointed posteriorly. The pieces of the set without cephalic ganglia regenerated from the posterior cut surface, but the new tissue grew out in a rounded mass and neither new nor old tissue ever acquired a tapering form. Various other methods of attacking the problem were employed, and all with the same result.

It was found that pieces of a certain shape would move in circles after the extensive contraction of the cut surface had taken place, this movement being due to the one-sided effect of the cilia. In such pieces the regeneration occurred as usual from the posterior cut surface; the new tissue, however, did not grow out in the direction of

the longitudinal axis of the body, but the direction of its growth depended entirely on the direction of movement of the piece. In this manner specimens were obtained in which the tip of the regenerated tail was overlapped by the head. The body was not simply temporarily bent, but it was impossible for the animals to straighten themselves. The new tissue had grown out at an angle to the longitudinal axis, the size of the angle varying inversely as the size of the circle in which the piece moved. In other words, the regenerating tail being used constantly for attachment was subjected to tension, but, owing to the circular movement of the piece, this tension was not in the direction of the longitudinal axis, but formed an angle with it. The tension was effective either directly or indirectly in determining the arrangement of the tissue.

All of these facts and many others observed show the great influence of mechanical factors in determining the form of these animals.

It may be said that in *Leptoplana,* as in *Stenostoma,* the characteristic form or outline of the body is determined by mechanical conditions of tension. In the normal environment, however, these conditions depend on the characteristic activity of the animal and the characteristic properties of its tissues. From this point of view the forms may be regarded as predetermined, but even so, only indirectly, since it is the result rather than the cause of function. These problems will be discussed more fully at another time: here it is possible only to call attention to them briefly.

II. Cestoplana

The species of *Cestoplana* employed was a small white form averaging about 10 mm. in length and less than 1 mm. in transverse diameter. A slightly differentiated head with a number of eyes was . present. Posterior to the cephalic ganglia the much-branched intestine filled the whole body: the pharynx was situated in the middle region of the body with some slight variation in position.

In general regenerative power this form differed widely from *Leptoplana.* In no case did extended growth in the posterior direction from a cut surface occur: the wound healed and a very small bud of new tissue appeared, but this was all. The result was the same whether only a small portion had been removed from the posterior end of the body or all except two or three millimeters at the anterior end.

From an anterior cut surface, anterior to the brain, new tissue

grew out, replacing the parts of the head removed. Moreover, in this form regeneration of the cephalic ganglia was possible.

The difference between this form and *Leptoplana* in this respect may perhaps be due, as has been suggested by others in connection with somewhat similar phenomena in other forms, to differences in the degree of cephalization of the nervous system.

Though *Cestoplana* does not give rise to a large amount of new tissue after injury, it completes regeneration in another way, viz., by the formation of the missing parts within the portions of the body still remaining. In pieces without a pharynx a new pharynx regenerates (with certain exceptions noted below) within the old tissue at greater or less distance from the end. Its position in any particular case depends on the region of the body from which the piece in question was taken. In pieces taken from the region anterior to the old pharynx the new pharynx regenerates in the posterior half of the piece. The further anterior the level which the posterior end of the piece represents, the nearer the posterior end is the new pharynx. In pieces posterior to the old pharynx, the new pharynx appears near the anterior end of the piece, provided this represents a level not far posterior to the old pharynx: in pieces from the extreme posterior end of the body, no pharynx is regenerated. I am inclined to believe that the position of the pharynx is determined by the movements and pressure of the internal contents: the grounds for this belief cannot be given at present. A somewhat similar, though not identical explanation has been offered by Bardeen for the position of the regenerating pharynx in *Planaria*.

The pieces of *Cestoplana* undergo a marked change in form, becoming relatively longer, more slender, and tapering posteriorly. Here, as in *Leptoplana,* the change in form can be shown to be due to the longitudinal tension to which the tissue is subjected in consequence of the antagonism between forward movement and attachment by the posterior end to the substratum. In the posterior portions of the body where this tension is greatest the elongation is often so great that the intestine is torn apart and undergoes disintegration.

One of the most interesting phenomena observed in this form was the disintegration and disappearance of portions of the intestine in specimens kept without food. In such specimens kept for a few weeks in clear water the branches of the intestine in the anterior and posterior regions of the body gradually lose their dark color and become indistinct. Examination under a high power shows that the tips of these branches are actually disintegrating; the cells and

granular masses are clearly visible in the region previously occupied by the branch and in the interstices of the parenchyma. In the course of two to three months this disintegration proceeds so far that in the terminal regions of the body only an unbranched axial intestine remains intact. Meanwhile the disintegration of the lateral branches is advancing from the terminal regions toward the middle region of the body: the branches in the pharyngeal region are the last to show traces of disintegration. This disintegration and disappearance of intestinal branches appear to be essentially atrophy as the result of disuse. In the absence of food the intestinal contents diminish in quantity, and in the course of time become insufficient to fill the whole intestine, even to a moderate degree. Those parts of the intestine which are least often expanded by contents, or from which the contents are most frequently forced out by contraction of the body, viz., the terminal regions, are the first to undergo atrophy. The atrophy gradually extends toward the middle region, those parts near the pharynx being the last affected. This fact indicates that Bardeen is correct in his belief that the pharyngeal region is a region of " greatest intestinal pressure." The axial intestine does not undergo atrophy, though it is often reduced in size, especially its terminal portions. Even after several months it contains a thick, dark, granular fluid, which is forced to and fro in it by the contractions of the body. Probably the stimulation of the intestinal walls by this fluid is sufficient to prevent disintegration of this part of the intestine.

A very interesting modification of this process of atrophy was observed in numerous pieces from the posterior region of the body, which were kept for four months or more. These pieces did not regenerate a head or pharynx: they showed little power of movement beyond peristaltic contractions, and there was no communication between the intestine and the exterior. In the course of about three months all lateral branches of the intestine had completely disappeared, leaving only a straight, unbranched axial intestine. Within the axial intestine in every case was found a fluid or semifluid residue, either of the intestinal contents, or of disintegrated portions of the intestinal wall, or of both. This residue, dark in color and filled with granules, is forced to and fro in the axial intestine by the contractions and movements of the piece. During all this time the piece is of course decreasing in size, but the dark residue in the axial intestine apparently does not decrease in quantity after a certain time. Undoubtedly all nutritive substances have been removed from the fluid long before this, and probably equilibrium as regards

diffusion has been established: there remain only waste products, débris, etc. Now, as the piece decreases in size a point is reached where the intestinal space is reduced to such an extent that the residual fluid fills it completely and begins to exert a pressure upon its walls. The changes following this period are most remarkable: from the sides of the axial intestine a new set of lateral branches begins to grow. These are typical intestinal branches, not ruptures or masses of disintegrating cells. The entrance into and exit from these branches of the residual fluid can be clearly observed. Moreover, they are not the old branches: it might be believed that the old branches had not actually disintegrated, but had merely become invisible as the result of collapse and were now become visible again because distended. This, however, is not the case; these new branches are less numerous and farther apart and much more delicate than the old branches, but they are unmistakably intestinal branches. This formation of a new set of intestinal branches was observed repeatedly in pieces of the kind described, which were kept for several months. It is of considerable importance as indicating how closely typical structure of this organ is dependent upon its typical function. So close is this dependence that typical structure cannot continue to exist when the typical function is abolished. One further point may be merely suggested here; viz., the possibility that the functional stimulus may be a mechanical tension exerted on the intestinal walls by the fluid contents.

III. CERIANTHUS

The body of *Cerianthus* is elongated and almost cylindrical in form, except aborally where it tapers to a blunt point. The course of regeneration, and indeed the possibility of regeneration, is determined to a large extent by the shape of the piece. In pieces cut from the body the body-wall soon begins to roll inward at the cut surface, this change being due not to muscular contraction, but to the elasticity of certain layers of the body-wall, apparently the mesoglœa, at least in large part. In cylindrical pieces inrolling of the ends only occurs and typical regeneration is possible. In pieces slit longitudinally or in strips cut from the body the inrolling may often be spiral or transverse and typical regeneration impossible.

In cylindrical pieces regeneration is typical and complete, resulting in a new individual of smaller size than the original and differing from it more or less widely in form. Complete regeneration is possible in pieces which comprise only a small fraction of the whole, provided they are in the form of rings of tissue. Such pieces, about

one twentieth of the body-length, have been observed to regenerate completely.

The rapidity of regeneration is greatest at the oral end and decreases aborally, until in a short aboral region complete regeneration does not occur. Apparently the power of growth and in general the reactive capacity of the tissues are greatest at the oral end and decrease aborally.

Rapidity and amount of regeneration are influenced by temperature, increasing with rise and decreasing with fall of temperature. This influence of temperature is clearly seen in the difference in the rapidity and amount of regeneration in summer and in winter, the temperature of the water being much lower during winter.

In pieces above the minimum, size has no effect on the rapidity of regeneration and only a very slight effect in the later stages upon the amount. In other words, the new parts are far from being proportional to the size of the piece from which they arise. A piece five millimeters in length produces tentacles of about the same length as a piece twenty or thirty millimeters in length. Only after several weeks does the smaller piece fall slightly behind the larger. These facts make it evident that porportionality is by no means retained in the regenerating pieces of *Cerianthus* and are therefore difficult to reconcile with those theories in which this proportionality plays an important part.

It was found to be possible to analyze to a certain extent the process of regeneration and to inhibit it experimentally. The more important results obtained from these experiments are given briefly in the following paragraphs.

As regards the growth of tissue from cut surfaces of the body-wall, it was found that new tissue arising from cut surfaces appears to obey the laws of capillarity to some extent. New tissue was never seen to grow out from a single exposed cut surface; the surface may heal over, but no further growth occurs. When, however, two cut surfaces are in contact, union between them occurs, and the growth thus initiated may continue in the form of a thin membrane with concave free margin for a certain distance between two diverging cut surfaces. The distance to which the membrane is capable of extending depends, in a given species of *Cerianthus,* on the angle of divergence of the cut surfaces—the greater the angle the less the distance over which the new membrane of new tissue can extend. It follows that if the angle of divergence be too great for the extension of this membrane, the wound cannot close. Thus, by preventing contact of cut surfaces or by fixing their position at a

certain angle, the closure of wounds can be experimentally pre-
vented or stopped at any point.   The relation between extent of the
new tissue between diverging cut surfaces and the angle of diver-
gence of these surfaces, as well as the concave free margin of such
new tissue are comparable with the behavior of fluid films under
similar conditions.   This new embryonic tissue certainly contains a
high percentage of water, and it is not at all improbable that the
laws of capillarity should determine its behavior to a greater or less
extent.

The second point of importance in the experimental analysis of
regeneration concerns the influence of water-pressure on regener-
ation.   As is well known, *Cerianthus* is essentially a hollow sac with
a mouth at one end ; the sac is divided peripherally into chambers by
the mesenteries ; a hollow marginal tentacle opens orally from each
one of these chambers, and most of them communicate also with a
smaller labial tentacle.   Under normal conditions, and when undis-
turbed, the body and tentacles of the animal are distended by the
water in the enteron, which is under a considerable degree of pres-
sure.   When a cut is made in the body-wall, or a piece is removed,
collapse of body and tentacles occurs at once.   As I shall show in
detail elsewhere, this is due simply to escape of water from the
enteron and not at all to a change in osmotic conditions, as Loeb[1]
believed.   Typical regeneration of pieces can never occur unless the
piece can in some manner acquire again the form of a sac in which
water can be retained under pressure.   If closure of the aboral end
of pieces be prevented by repeated artificial separation of the cut sur-
faces, the appearance of the tentacles at the oral end is delayed.   If
the piece be allowed to close until small tentacle-buds have ap-
peared, and then be opened at the aboral end and kept open, growth
of the regenerating tentacles ceases, and they may even decrease in
size.   In every case the delay or inhibition continues as long as an
opening is present which prevents distension of the body by water
and pressure upon its walls.   As soon as the openings are allowed
to close, the interrupted regeneration continues.   The objection may
be made that the pressure of the water in the enteron will be the
same in all regions of the body, and that it cannot account, therefore,
for the localization of organs at certain regions.   To this the answer
may be made that currents in definite directions are caused in the
enteron by the vibrations of cilia and that these currents striking
the wall at certain points may produce characteristically localized
differences in pressure.   It is highly probable, as I shall show else-

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[1] *Untersuchungen zur physiologischen Morphologie,* I, 1891.

where, that the position of regenerating tentacles is determined in this manner.

It is possible also to reduce the fully grown tentacles of normal animals to mere stumps by keeping the aboral end of the body continuously open. After the tentacles have remained collapsed for a time, the tips begin to shrivel and are gradually absorbed until only stumps remain. If, now, the aboral end of the body be allowed to close, the enteron once more becomes distended with water, and the tentacles, being again distended, gradually grow out again. Many other instances of the close relation between growth and the pressure of the water in the enteron were observed, but their description must be deferred.

The chief results of my work on *Cerianthus* may be summed up in the statement that regeneration in *Cerianthus* is influenced in large measure by simple mechanical conditions of pressure and tension, and that in the absence of these conditions in typical amount and localization, typical regeneration is impossible.

IV. Tubularia

A part of the work on *Tubularia* consisted of the reëxamination of certain regulative phenomena described by other investigators. My conclusions differ from theirs in various points, but a critical discussion may be omitted here.

A few points of interest which are either new in themselves or afford new interpretations of known facts may be mentioned briefly. It was found that in many instances the pieces cut from the stems of *Tubularia* would produce a stolon at the aboral end, and if they were in contact with a solid body would become attached in a few days. In many cases these stolons attained a length of fifteen millimeters or more and frequently became branched. In the course of time most of the branches of the stolon, and often even the tip of the main stolon, turned upward away from the substratum and developed new hydranths in the usual manner. If the stolon did not come into contact with a solid surface within a day or two after its formation, the end usually produced a new hydranth in a much shorter time than when it succeeded in attaching itself to a surface. This power to produce stolons is found only in the more vigorous stems, and long pieces are more likely to produce stolons than short pieces.

The formation of a hydranth at both oral and aboral ends of pieces from the stem of *Tubularia,* or the formation of a hydranth at the oral end and nothing at the aboral end, have been described by a

number of authors as the usual methods of regeneration. In case two hydranths are formed, the oral appears earlier than the aboral. The formation of stolons at the aboral end in vigorous pieces, as described above, indicates that the aboral end of the piece is more or less "determined" for stolon-formation. It is probable, therefore, that the delay in the formation of the aboral hydranth in pieces which have not formed a stolon is due to the time required for the changes preparatory to hydranth-formation instead of stolon-formation. The production of stolons by certain pieces and the production of hydranths from the ends of the stolons when attachment is impossible, or much later, after the stolon has become attached, appear to be reactions to unfavorable conditions. Only vigorous pieces form stolons at all; *i. e.,* in pieces less vigorous the stimulus to hydranth-formation overcomes the stimulus to stolon-formation before the latter becomes effective. Now, in the vigorous pieces which have produced stolons, the stolon itself, when subjected to unfavorable conditions, develops a hydranth at its tip. Somewhat similar phenomena have been observed in other hydroids.

An extended study was made of the proportions of both normal and regenerated hydranths from various regions of the stem: this included measurements of regenerating hydranths before their emergence from the perisarc, as well as of fully-developed hydranths. At least four different dimensions were measured in each case. Comparison of the measurements of hydranths from different regions shows that their proportions are characteristically different. Hydranths arising from the extreme oral end of a stem possess different proportions, both before and after emergence, from those arising at the oral end of a piece from the middle or basal portion of the stem. The proportions of aboral hydranths are different from those of oral hydranths, and also differ among themselves, according to the region from which they arise. The form of the hydranth is then not the same under these different conditions of origin: on the contrary, there are characteristic differences corresponding to the different conditions.

These facts indicate that the various conditions play a part in determining the form of the result in each case. Discussion of their theoretical bearing is reserved until the data can be given in detail.

In conclusion I desire to express my most sincere thanks to the Smithsonian Institution for the opportunity afforded me of carrying on the work above described.