

An Analysis of Form-Regulation in Tubularia.

I. Stolon-Formation and Polarity.

By

C. M. Child.

Eingegangen am 5. Januar 1907.

Some fourteen years ago LOEB ('92) observed that the oral ends of pieces of *Tubularia mesembryanthemum* usually gave rise to hydranths when not in contact with the substratum. Pieces so placed that the aboral ends were surrounded on all sides by water produced almost without exception hydranths at these ends. But if the aboral ends were brought into permanent contact with the surface of a solid body stolons frequently appeared. Stolon-formation was frequently observed when the pieces were laid on the bottom of the containing vessel, but LOEB states that the vessel must remain undisturbed and the same side of the piece must remain in contact with the substratum in order that this result may occur.

MORGAN and STEVENS ('04) have recently observed in experiments on *Tubularia marina* that this species as compared with others exhibits a »stronger tendency to produce a stolon from an aboral cut end rather than a heteromorphic hydranth«.

While at Naples in 1902/3 I noted the frequent development of stolons at the aboral ends of pieces of *T. mesembryanthemum* and reached conclusions somewhat different from those of LOEB regarding the influence of contact. During the last two winters further experiments along the same line were performed at the Hopkins Laboratory, Pacific Grove, California, this time on *T. marina*.

The present paper includes an account of my observations and experiments regarding stolon-formation on both species together with some consideration of their bearing upon the problem of polarity in *Tubularia*.

I. Stolon-Formation in *Tubularia mesembryanthemum*.

1. The Frequency of Stolon-Formation.

My records as regards stolon-formation are incomplete in certain respects because I failed to recognize the full significance of some of the results until it was too late to obtain further data by additional experiments, but they are sufficient to permit certain important conclusions.

The first point for consideration is the frequency of stolon-formation during regulation in this species. The following table (Table I) shows the results obtained. All pieces used in these experiments were cut from stems 15 mm. or more in length. The so-called whole stems include all that part of the stem between a point 2 mm proximal to the original hydranth and the stolon. Half-stems are the distal or proximal halves of stems similarly prepared, the original hydranth and about 2 mm. being removed in all cases from the distal end. No piece in these series was less than 10 mm. in length.

Table I.

Part of Stem included	No. of Pieces	No. of Stolons	%
Whole stems	46	24	52
Distal pieces more than half but less than whole length of stem	75	19	25 +
Proximal halves	16	7	44 —
Distal halves	16	5	31 +
Distal pieces of less than half but more than one fourth the length of the stem	40	12	30
Totals	193	67	35 —

This table shows that in pieces from one fourth to the whole length of the stem 35% gave rise to stolons. It should perhaps be added that stolon-formation occurred in all cases at the aboral end of the piece.

2. Stolon-Formation in Different Regions of the Stem.

We may now consider the frequency of stolon-formation in different regions of the stem. Table I shows that the percentage of stolons is highest in whole stems and proximal halves, i. e. where

the cut surface from which the stolon arises is at the extreme proximal end of the stem. Table II shows the number and percentage of stolons formed at the proximal end and elsewhere.

Table II.

Region of Aboral Cut Surface	No. of Pieces	No. of Stolons	%
Extreme aboral end of stem	62	31	50
Other regions	131	36	27.5 —

According to this table the frequency of stolon-formation at the extreme proximal end of the stem is almost double the frequency in other regions.

3. Stolon-Formation and the Physiological Condition of the Stem.

When we attempt to compare the relative frequency in other regions the figures given in Table I indicate that stolons appear less frequently in pieces which include more than the distal half of the stem (25) than in pieces including only the distal half or less (31, 30). We should expect to find the frequency of stolon-formation decreasing rather than increasing with increasing distance of the aboral end of the piece from the proximal end of the stem. I believe, however, that the discrepancy in Table I is only apparent. During my experiments I often observed that stolons seemed to appear much more frequently when the stems were cut within a few hours or a day or two after they were collected than when the colonies were allowed to stand in aquaria for several days before using, even though they were in flowing, well-aerated water. It is well known that colonies of *Tubularia* kept in aquaria lose the hydranths after a few days. This is an indication of a change in physiological condition.

In the various series of experiments which form the basis of Table I all of the half-stems and smaller pieces were cut within a few hours after collecting but the whole stems and the pieces of more than half-length were cut at various times up to four days after collection. Thus it is possible to determine for these pieces whether the time elapsing between the removal of the colony to the laboratory and the operation affects the frequency of stolon-formation. The results are given in Table III.

Table III.

Time between collection and operation	No. of Pieces	No. of Stolons	% of Stolons
1—6 hrs.	31	21	68 —
1—2 days	45	17	38 —
4 days	20	1	5

While the number of cases is probably too small to give the percentages an absolute value, the differences are too great to be the result of error or of chance differences in the stems or colonies. Moreover, these results confirm a large number of observations in which accurate records were not kept.

The results obtained in Table III enable us to reduce the apparent discrepancies in Table I. Twenty of the pieces in the second group of Table I, i. e., the pieces of more than half and less than whole length were cut after the colonies had stood four days in the aquarium. A stolon was formed in only one case among these twenty pieces. The remaining 55 pieces were cut from the colonies after these had stood from one to two days and 18 stolons were formed, i. e., about 33%. On the other hand all of the half-stems and smaller pieces, i. e., the last three groups and also most of the whole stems were cut within a few hours after collection of the colonies. If the length of time elapsing between collection and the operation influences the frequency of stolon-formation, it is evident that the frequencies given in Table I are not directly comparable since the pieces were not all in a similar condition. As compared with the results on stems cut within a few hours after collection the frequency of stolon-formation in the first group of Table I is too low since a part of the stems were cut from the colonies after two days: in the second group, pieces of more than half-length, the frequency is much too low because most of these pieces were cut two days and twenty of them four days after collection: but in the last three groups all pieces were cut within a few hours after collection and the figures are therefore comparable. These indicate that proximal half stems give rise to stolons much more frequently than distal halves.

As is indicated by the loss of the hydranths physiological changes are going on in tubularian colonies kept in aquaria. It is impossible at present to determine the exact nature of these changes but I am inclined to believe that they indicate a decrease in energy. Food is certainly far less abundant in the water of the aquarium

than under natural conditions. It is very probable, however, that other factors such as differences in the constitution of the sea-water and perhaps other factors unrecognized as yet play a part.

But whatever the nature of these changes it is evident from the data given above that the frequency of stolon-formation is greatest when the physiological condition is most nearly normal and decreases as the condition departs more and more from the normal.

Further investigation along this line is desirable and care must be taken to eliminate the time-factor in examining the regional frequency of stolon-formation and on the other hand to eliminate the regional factor in examining the influence of time. I hope to offer further data in the future but since my work will probably be based on other species of *Tubularia* it has seemed advisable to present the data already at hand.

Another fact which accords with the conclusions reached regarding stolon-formation and physiological condition is the fact that stolon-formation is never long delayed. If a stolon does not appear within two or three days after section of the stem it is very unlikely to appear at all. In most cases observed the stolon was well advanced within forty-eight hours after section. These results like those cited above show very clearly that the change in physiological — probably nutritive conditions during the experiment very soon renders the formations of stolons impossible.

4. Stolon-Formation and the Length of the Piece.

The pieces used in these experiments all include more than one fourth the length of the stem and none was less than 10 mm. in length. In pieces of less than one fourth the whole length stolon-formation has been observed very rarely although hundreds of such pieces have been examined. Even short pieces from the extreme proximal regions of the stem almost always give rise to hydranths or nothing at the aboral end. Sometimes a slight bulging or outgrowth of the coenosarc which might perhaps be regarded as the beginning of stolon-formation is observed before the hydranth appears, but this does not continue and a hydranth usually appears sooner or later. Even in the proximal regions of the stem stolons have been observed in short pieces in only 2—3% of the cases. In the distal regions, i. e., in the distal half or two thirds the formation of stolons has never been observed in short pieces, except in two stems one of which was cut into eleven pieces as nearly equal in length as possible

and averaging 1.5 mm. The first four pieces in succession from the distal end gave rise to hydranths or nothing at their aboral ends while all the rest — seven in number — gave rise to stolons varying in length from 0.5—1 mm. and six of these became firmly attached to the glass of the vessel which contained them. In the other case a similar stem was cut into ten pieces and stolons appeared on two pieces from the proximal region. These two cases are of interest as indicating that the internal conditions which bring about stolon-formation are much more intense and extend further distally in some stems than in others.

The fact that stolons are so very rare in short pieces from any region indicates that the length of the piece constitutes a factor in stolon-formation. It is not the absolute length of the piece that is most important but its relative length, i. e., the proportion of the whole stem which it includes. Whole stems 10—15 mm. in length produce stolons almost as frequently as stems 60 mm. in length, Doubtless in short stems the absolute length is of some importance in this connection, but in general the relative length of the piece with respect to the stem from which it is taken is much more important than its absolute length.

5. Stolon-Formation and Contact.

According to LOEB ('92) stolons rarely appear in *T. mesembryanthemum* unless the aboral ends are in contact with some solid object. My own experiments do not confirm this conclusion. Of the 67 stolons which appeared in the experiments cited in Table I, 27 were never in contact with a solid object, 26 were attached at some time during their growth and in 14 cases no record was kept of attachment. LOEB regards the aboral end as in contact when the piece is laid flat on the bottom of a vessel, but this is often not the case. If the stem is bent it often lies so that the aboral end is raised above the surface of the glass, yet I have seen stolons arise in many such cases. Moreover, in a considerable number of experiments the aboral ends of stems were raised by placing another stem or some other object beneath the stem at some distance from the aboral end and in these cases also stolons appeared about as frequently as when the stems were lying flat on the surface. In many cases also the stolons were freed soon after they became attached and the stems were placed so that they could not again come into contact with the surface yet the stolons continued to grow. In other cases the sto-

lons were allowed to attach themselves again and were again freed, this being repeated several times in some cases.

These results lead me to believe that internal conditions are of chief importance in determining stolon-formation. Undoubtedly contact with a solid object favors stolon-formation but in many cases it is certainly not a determining factor. Moreover, it seems to me that the aboral end of a piece can scarcely be said to be in contact when the piece is laid on the flat surface of a glass or other vessel. Even if the shape of the stem is such that the aboral end touches the glass it is only the perisarc that is in contact. The coenosarc must extend beyond the cut end of the perisarc before it can come into contact with the glass. When a stolon is formed in such cases it soon attaches itself to the glass but even in cases where the aboral end is slightly raised the stolon may bend downward and attach itself. It seems probable therefore that many of the cases of stolon-formation observed by LOEB where the pieces were lying flat on the surface of the dish were determined not by contact but by internal conditions, the attachment taking place only after the stolon had begun to form. Further experiments upon the influence of contact on stolon-formation are needed.

6. The Length and Form of the Stolons.

No complete record of the length of all stolons observed was kept, but many were measured repeatedly. The maximum length varied in different cases from 0.3 mm to 11 mm. The most common form observed was a simple straight outgrowth which often became slightly enlarged and rounded at the tip. In many of the whole stems, however, the stolon, though at first simple, soon became more or less highly branched and each branch continued to grow for some days. Branched stolons and stolons more than 2—3 mm. in length were rarely observed except in long, whole or nearly whole stems, but here they were frequent. In such long, whole or nearly whole stems the growth of the stolon continued during a much longer period than in shorter stems and in half-stems. In many cases observed the stolons arising from long stems continued to grow during twelve days, while in short stems and half-stems growth almost always ceased after three or four days or earlier.

All of these facts regarding the occurrence of long and branched stolons and length of the growth-period indicate a correlation between the growth of the stolon and the amount of material available in the

piece. Other things being equal the greater the amount of material in the stem the greater the growth of the stolon. Certain other general observations may be mentioned in this connection. Stolon-formation was more frequent and the stolons longer in pieces from colonies which appeared to be in vigorous condition than in those from colonies in poor condition. Conclusions regarding the vigor and general condition of different colonies are necessarily somewhat uncertain but colonies in which all or nearly all the stems bear good-sized hydranths with numerous gonads may I think be regarded as in better condition than those with small hydranths, few gonads, and many stems without hydranths or with degenerating hydranths, and it was upon these points that my conclusions regarding vigor were based. I believe we are justified in concluding that stolons are likely to appear more frequently and grow to a greater length in stems that are well nourished and otherwise in good physiological condition than in those that are poorly nourished or otherwise in poor condition.

7. The Formation of Hydranths at the Tips of Stolons.

LOEB ('92) and others who have observed stolon-formation in *Tubularia* have noted the frequent formation of hydranths at the tips of the stolons. Often the end of the stolon assumes a vertical position before the hydranth is formed, but sometimes it is only the short new stem of the hydranth which assumes this position after it emerges from the perisarc of the stolon.

Among the 67 stolons of Table I, 38 sooner or later gave rise to hydranths at their tips. The branched stolons sometimes gave rise to hydranths at the tips of two or even three of the branches. The hydranth at the tip of the stolon never appears until the stolon has ceased to grow: it follows therefore that the longer the period of growth of the stolon the later the appearance of the hydranth, and further that the hydranths at the tips of the stolons appear later in whole stems and relatively long pieces than in shorter pieces, for the growth-period of the stolon is longer in the relatively long pieces. I was unable to determine with certainty whether a relation exists between attachment or non-attachment of the stolons and hydranth-formation. Among the 38 cases of hydranth-formation hydranths appeared at the tips of unbranched stolons which had never been in contact in 16 cases and at the ends of unbranched attached stolons in 9 cases. These figures seem to indicate that hydranths appear more frequently at the tips of stolons which have not been attached,

and this might perhaps be expected, but the number of cases is too small to permit certain conclusions. Where branched stolons appeared all branches were attached in some cases while in others only a part were attached, the others being free. Among such stolons hydranths arose from attached branches in 5 cases and from unattached branches in 4 cases. These cases therefore do not permit any definite conclusions.

The fact that hydranths appear at the ends of stolons only after the latter have ceased to grow is I believe of some importance as indicating that the formation of hydranths is possible under conditions which do not permit the growth of stolons. The facts cited in several of the preceding sections seem to indicate that stolon-formation requires a relatively vigorous or well nourished condition. On the other hand it is known that pieces of the stem of *Tubularia* may continue to produce hydranths for several weeks after their removal from the colony and in almost total or total absence of nutrition from without. As a result of my observations I wish to suggest that a vigorous physiological condition with abundant nutritive material available favors stolon-formation at the aboral end while hydranth-formation is possible under the opposite condition.

If this suggestion is correct it is not difficult to understand why stolons appear more frequently and are longer in relatively long than in relatively short pieces and also why the frequency of stolon-formation decreases as the time during which the colonies are kept in aquaria before operation increases.

II. Stolon-Formation in *Tubularia marina*.

While I am not able to confirm the opinion of MORGAN and STEVENS ('04) that *T. marina* exhibits a »stronger tendency to produce a stolon from an aboral cut end rather than a heteromorphic hydranth« my experiments show that stolons are of common occurrence in this species.

In pieces of less than half the length of the stem only very slight outgrowths of doubtful nature have been observed. The results for whole and half stems are given in the following table (Table IV).

Here the percentage of stolons is almost the same for whole stems and for proximal halves, but in the distal halves the frequency of stolon-formation is less than half as great. A larger number of

Table IV.

Nature of Pieces	No. of Pieces	No. of Stolons	% of Stolons
Whole stems. . . .	45	19	42.2
Distal halves. . . .	27	5	18.5
Proximal halves . .	22	9	40.9

cases might alter the percentages more or less, but would probably not alter the relations in general. In all of these cases the stems were cut within a few hours after collection so that the time factor does not enter. The stolons produced, especially those at the proximal ends of stems, were in general much shorter than those observed in *T. mesembryanthemum* and were all unbranched. These differences are probably due to the fact that in *T. marina* the coenosarc at the proximal end of the stem is much thinner and more slender than in *T. mesembryanthemum* and hence there is less material available for growth there in that species. A hydranth was formed at the end of a stolon in only one case, a distal half included in Table IV: Attachment of the stolons to the substratum was observed in a number of cases but a complete record in regard to this point was not kept.

III. Experimental Increase in Frequency of Stolon-Formation.

In his earlier experiments on *Tubularia* LOEB ('92) discovered that new hydranths developed more rapidly and with longer stems in dilute than in normal sea-water. More recently SNYDER ('05) found that dilution of the sea-water up to a certain limit — between 50 and 60% increased the percentage of aboral hydranths in pieces of *T. crocea*.

In the course of some experiments with dilute sea-water I found that stolon-formation also seemed to occur more frequently in dilute than in normal sea-water. This was observed in a number of series only one of which is given below. As I hope to consider elsewhere the effect of dilute sea-water on *Tubularia*, only the results which concern us here are given in the tables. Unfortunately I was forced to conclude my work with a smaller number of experiments than might be desirable but the results are so striking as to leave no doubt regarding the effect of the dilute sea-water.

In Table V a series of experiments on *T. marina* performed at the Hopkins Laboratory, Pacific Grove, are given. 15 whole stems,

15 distal half-stems, and 15 proximal half-stems were placed in 60% sea-water and the results recorded.

Table V.

Nature of Pieces	No. of Pieces	No. of Stolons	% of Stolons
Whole Stems . . .	15	14	93.3
Distal halves . . .	15	8	53.3
Proximal halves . .	15	12	80

The whole stems and half stems included in Table IV serve as controls. For ease in comparison the percentages of stolons in Tables IV and V are given in Table VI.

Table VI.

Nature of Pieces	% of Stolons in normal sea-water	% of Stolons in 60% sea-water
Whole stems. . . .	42.2	93.3
Distal halves. . . .	18.5	53.3
Proximal halves . .	40.9	80

For whole stems and proximal halves the percentage of stolons is about doubled in 60% sea-water while for the distal halves it is nearly tripled.

In my records of experiments on *T. mesembryanthemum* at Naples in 1902 I find somewhat similar results. When the experiments were performed I did not recognize their significance and so did not carry them further. In this case eight distal and eight proximal halves from the same stems were placed in 75% sea-water and a similar series from the same colony in normal sea-water for control. The results are given in Table VII.

Table VII.

Nature of Pieces	% Stolons in normal sea-water	% Stolons in 60% sea-water
Distal halves. . . .	0	37.5
Proximal halves . .	0	50

Probably more extended experiments would alter the percentages more or less for both species but the results as they stand are quite

sufficient to show that dilution of the sea-water has a marked effect in increasing the frequency of stolon-formation.

But in order to make the case clear it is necessary to consider briefly the nature of the effect of dilute sea-water on *Tubularia*. The work of LOEB has indicated that the effect is in general an acceleration of growth processes. It cannot be supposed, however, that dilute sea-water produces a localized qualitative change at the aboral ends of pieces of such a nature as to alter the specification of this region from hydranth-formation to stolon-formation. The only possible conclusion is that conditions or processes already present are either directly or indirectly increased in energy or accelerated. In other words dilution of the sea-water simply increases the frequency of outgrowth from the aboral end but cannot be supposed to determine its character. These experiments force the conclusion that the aboral ends of pieces of *Tubularia* are usually and probably always physiologically predetermined or specified in the direction of stolon-formation, at least in regions proximal to the middle of the stem and probably also to a less degree in those regions distal to it. Under the usual experimental conditions the internal factors are in most cases not sufficient to bring about the actual outgrowth of a stolon except in vigorous well nourished stems. But in dilute sea-water they become sufficient to bring about a visible result in a much larger percentage of cases.

Objections to this interpretation may be raised on the ground that this outgrowth is merely an outpushing of the coenosarc in consequence of osmotic conditions accompanying dilution of the medium. If these outgrowths are purely osmotic phenomena we should expect them to appear at both ends of the stem. Occasionally irregular masses of coenosarc are found protruding from one or both ends of the stems in dilute sea-water, but these are very different from the stolons, being in most cases masses of rounded or irregular form, much larger in diameter than the stem and not elongated and frequently almost solid. These appear to be passive outpushings of the coenosarc in consequence of osmotic conditions: after a few hours they often collapse or are reduced in size.

The stolons, however, differ in appearance from these passive outpushings. Their diameter is the same or almost the same as that of the stem from which they arise; they usually form a direct continuation of the stem at least for some distance; the layers of the coenosarc and the enteric cavity are clearly visible in them: and

finally if they are near enough to the substratum they become attached, provided the stem is not moved about. They are clearly typical stolons, not passive outpushings of the tissue.

Moreover, we have a basis in other phenomena for the belief that the dilution of the medium increases quantitatively the energy of reaction in *Tubularia*. Various authors have noted that withdrawal of water from protoplasm decreases its irritability and vice versa up to a certain point. In encysted or other quiescent stages the percentage of water is usually low. We are justified therefore in concluding that the effect on *Tubularia* of dilution of the medium is something more than a mere osmotic increase of turgor and internal pressure. Additional data bearing on this point will be presented later.

IV. Polarity in *Tubularia*.

The experiments described above are important in that they place the question of polarity in *Tubularia* in an entirely new light. DRIESCH, MORGAN and others who have worked with *Tubularia* have paid little attention to stolon-formation as an indication of polarity in this form. Only recently DRIESCH ('05, p. 695) has reaffirmed his views in the following statement regarding *Tubularia*. »Es ist nun hier der richtige Ort die allgemeine Bemerkung einzuschalten, daß ganz im unbestimmten und allgemeinen bei einem Organismus, der stets ohne weiteres¹⁾ an seinen beiden freien Enden Polypen restituieren kann, natürlich überhaupt nicht wohl von ‚Polarität‘ und deren ‚Umkehr‘ geredet werden kann. Im allgemeinen Sinne ist hier eben keine Polarität vorhanden, nur im spezifischen, z. B. auf die Größe oder Geschwindigkeit eingeschränkten Sinne, hat das Wort hier Bedeutung.«

It will be sufficiently evident from the proceeding sections that *Tubularia* does not »stets ohne weiteres« produce polyps at both ends. Even in normal sea-water the percentage of stolons is too great to be neglected and in dilute sea-water it is greatly increased.

The experimental data force us to the conclusion that a »true polarity« exists in *Tubularia*. Failure to recognize its existence has been due to the fact that the process of stolon-formation can occur only under conditions which are often not present in isolated pieces of stems.

¹⁾ Italics my own.

But the failure of the stolon to form does not explain why a hydranth should appear in its place in so many cases. In order that this may occur not more by a quantitative but a qualitative change of specification at the aboral end must take place. The factors concerned in this change will be considered elsewhere. I believe, however, that both external and internal factors may be concerned.

But however the change may be brought about it is evident that the formation of the hydranth at the aboral end in pieces of *Tubularia* is essentially a secondary process which has nothing to do with the original polarity.

Before proceeding farther it is necessary to take definite position regarding the nature of polarity in general. All the facts seem to me to indicate that the process of hydranth- or stolon-formation like other »formative« processes is essentially a dynamic reaction or reaction-complex and that it is not associated with peculiar »formative substances« of mysterious and unknown nature. This reaction-complex is not necessarily localized alone where the structure appears; it may merely be most intense there or the simultaneous occurrence of other reaction-complexes in adjoining regions, or other special conditions may determine the localization of the structure, as I shall attempt to show in connection with other experiments. The character and energy of the characteristic reaction-complexes in different regions must depend at least in part upon the physiological or functional relations of the various parts. There can be no doubt, for example, that those regions of the stem nearest the original hydranth are much more directly involved in »hydranth-processes« and reactions than in »stolon-processes« and the reverse is true of the proximal regions of the stem.

Moreover, it is not impossible that stimuli of certain kinds pass chiefly or wholly in certain directions in the stem. Such stimuli may be factors in polarity.

But since the present relations are the result of past relations the problem of polarity becomes in part historical. Even if polarity should prove in the final analysis to be a fundamental and necessary property of protoplasm it would still be essentially a matter of dynamic relations of parts. But conclusive evidence that it is such a fundamental property has not as yet been presented and the fact that polarity is readily altered by changes in external conditions constitutes strong evidence for the opposite view.

Provisionally, at least, then, we may regard polarity as an axial

difference in the character and energy of reaction resulting from past physiological relations of parts to each other and to the environment. Polarity may be indicated by quantitative as well as by qualitative axial differences: unless we admit this we are in danger of failing to recognize the essential similarity of various regulatory phenomena.

On this basis we may interpret the delay in the formation of the aboral hydranth as representing the time necessary for the change in specification from stolon- to hydranth-formation. The length of this time must depend in some degree on the conditions in the different regions but factors of other kinds are also involved. It is a well known fact that the aboral hydranth appears earlier when the oral hydranth is prevented from forming. It is evident then that conditions at one end may influence those at the other. I have obtained very strong evidence — not yet published — that the attachment and stolon-formation at the aboral end accelerates hydranth-formation at the oral end in *Corymorpha*. It appears that in *Tubularia* the formation of the hydranth at the oral end retards the change in specification from stolon-formation to hydranth-formation. This retardation is probably, however, indirect rather than direct, i. e., the formation of a hydranth at one end increases the energy of stolon-forming factors at the other and so increases the time necessary to establish the hydranth-specification. A similar interpretation will probably hold for the fact discovered by KING ('04) that in *T. crocea* the presence of aboral hydranths delays the formation of oral hydranths. In this case the presence of the aboral hydranth probably renders the original oral end to some extent physiologically aboral. In general I have found that such influence of one end upon the other appears to be more marked in short than in long pieces. In certain cases special conditions alter the result: e. g., DRIESCH ('99) has shown that aboral hydranths usually appear earlier at the extreme proximal end of the stem than at the middle. In following papers a more extended consideration and analysis of the factors involved in determining the formation of the aboral hydranth will be given. Discussion of special cases is therefore postponed. The purpose of the present paper is to show that the aboral end originally possesses in greater or less degree a stolon-specification, and that the formation of the aboral hydranth is the result of a secondary change in specification, which occurs after the isolation of the piece.

Summary.

1) In pieces of *Tubularia mesembryanthemum* ranging from about one fourth to the whole length of the stem stolons are formed at the aboral end in approximately one fourth to one half of the cases.

2) In general the greater the length of the piece in proportion to the whole length of the stem the greater the frequency of stolon-formation. In half-stems stolons arise more frequently from proximal than from distal halves.

3) Short pieces of less than one fourth of the length of the stem from any region rarely give rise to stolons.

4) In *Tubularia marina* the conditions as regards stolon-formation are very similar to those in *T. mesembryanthemum*.

5) Stolon-formation occurs much more frequently in stems or pieces from vigorous colonies than from those in poor condition. The longer the period during which the colonies are kept in aquaria before the stems or pieces are isolated the more rarely are stolons formed. Stolon-formation always occurs early in the course of the experiment if at all. Stems or pieces which do not begin to produce stolons within two or three days after section very rarely produce them at all. These facts seem to indicate that relatively abundant nutrition or vigorous physiological condition in general is necessary for stolon-formation.

6) Hydranths appear at the ends of stolons only after the latter have ceased to grow. The longer the period of growth of a stolon the later the appearance of the hydranth at its tip. In general, stems and pieces are capable of producing hydranths long after they have become incapable of producing stolons. Various other facts such as the formation of new hydranths in nature at the ends of stolons, the continued regulatory production of hydranths until exhaustion occurs, the fact that short pieces almost always produce hydranths alone, very rarely stolons, etc. show clearly that the formation of a hydranth can occur under physiological conditions in which the formation of a stolon is impossible.

7) By dilution of the sea-water it is possible to increase the frequency of stolon-formation from two to three times. In whole stems of *T. marina* in 60 % sea-water the observed frequency of stolon-formation is 93 %, in proximal halves 80 %, and in distal halves 53 %.

8) Since it cannot be supposed that dilution of the sea-water

alters qualitatively the internal conditions at the aboral end alone we must conclude that this region is predisposed to stolon-formation in very many cases in which stolons do not appear in normal seawater. When we consider that stolon-formation apparently requires abundant nutrition or vigorous condition we are justified in concluding that the aboral ends of all except perhaps very short pieces are predisposed to stolon-formation but do not, in many cases, give rise to stolons because general physiological conditions do not permit the outgrowth of the stolon.

9) During the course of the experiment the original specification of the aboral end as a stolon-forming region gradually changes and it becomes a hydranth-forming region. According to this view hydranth-formation at the aboral end is a secondary process having nothing to do with the original polarity but induced by the change in physiological condition during the experiment. Hence the delay in the formation of the aboral hydranth represents the time necessary for the change in the specification. Various conditions such as the length of the piece, the presence or absence of a hydranth at the oral end, etc. may increase or decrease the length of this time.

10) Observation and experiment indicate that the original specification of the aboral end of a piece as a stolon-forming region is most firmly established and the reactions involved possess the greatest energy in the proximal regions of the stem and least in the distal regions with all intermediate conditions between. The original specification of the oral end as a hydranth-forming region apparently follows the reverse course. If this is the case *Tubularia* possesses a »true polarity«. The original polarity is, however, often masked in consequence of physiological changes during the course of the experiment.

Hull Zoological Laboratory, University of Chicago,
December, 1906.

Zusammenfassung.

1) An Stücken von *Tubularia mesembryanthemum*, die zwischen ein Viertel der Länge bis zur ganzen Länge des Stammes betragen, bilden sich Stolonen am aboralen Ende in nahebei einem Viertel bis zur Hälfte der Fälle.

2) Im allgemeinen ist die Frequenz der Stolonenbildung um so höher, je größer die Stücklänge im Verhältnis zur ganzen Stammlänge ist. An halben Stämmen erheben sich häufiger Stolonen an proximalen, als an distalen Hälften.

3) Kurze Stücke von weniger als ein Viertel Stammlänge von irgend welchem Bezirk dienen nur selten Stolonen zum Ursprung.

4) Bei *Tubularia marina* liegen die Verhältnisse bezüglich der Stolonenbildung im allgemeinen ähnlich wie bei *T. mesembryanthemum*.

5) Stolonenbildung kommt an Stämmen und Stücken von kräftigen Kolonien viel häufiger vor als an solchen von dürtigen. Stolonen bilden sich umso seltener, je länger die Zeit ist, während welcher die Kolonien bis zur Isolation der Stämme oder Stücke im Aquarium gehalten wurden. Stolonenbildung tritt, wenn überhaupt, immer frühzeitig im Verlaufe des Versuchs auf. Stämme oder Stücke, welche mit der Stolonenerzeugung nicht binnen 2 oder 3 Tagen nach dem Schnitt anfangen, bringen solche nur sehr selten noch hervor. Diese Tatsachen scheinen darauf hinzuweisen, daß eine verhältnismäßig üppige Ernährung oder eine sehr kräftige physiologische Beschaffenheit zur Stolonenbildung im allgemeinen notwendig ist.

6) Hydranten erscheinen an den Stolonenenden erst, nachdem das Wachstum der letzteren aufgehört hat. Je länger die Wachstumsperiode eines Stolonen ist, desto später erscheint die Hydrante an seiner Spitze. Im allgemeinen sind Stämme und Stücke noch fähig zur Hydrantenerzeugung lange, nachdem sie zur Stolonenerzeugung unfähig geworden sind. Verschiedene andre Tatsachen: die Bildung neuer Hydranten an den Stolonenenden in der freien Natur, die kontinuierliche, regulatorische Hydrantenbildung bis Erschöpfung eintritt, der Umstand, daß kurze Stücke fast immer nur Hydranten hervorbringen und nur sehr selten Stolonen usw., zeigen klar, daß die Hydrantenbildung noch unter physiologischen Bedingungen eintreten kann, unter denen die Stolonenerzeugung unmöglich ist.

7) Durch Verdünnung des Seewassers ist es möglich, die Frequenz der Stolonenbildung auf das zwei- bis dreifache zu erhöhen. An ganzen Stämmen von *T. marina* in 60%igem Seewasser beträgt die beobachtete Frequenz der Stolonenbildung 93%, an proximalen Hälften 80%, an distalen 53%.

8) Da man nicht annehmen kann, daß die Verdünnung des Seewassers die inneren Bedingungen nur am aboralen Ende qualitativ verändert, so müssen wir daraus schließen, daß diese Region zur Stolonenbildung in sehr vielen Fällen besonders disponiert ist, in denen Stolonen im normalen Seewasser nicht erscheinen. Wenn wir erwägen, daß die Stolonenbildung anscheinend überreichliche Nahrung oder kräftige Beschaffenheit voraussetzt, so sind wir zu dem Schluß berechtigt, daß die aboralen Enden aller Stücke, sehr kurze vielleicht ausgenommen, zur Stolonenbildung besonders disponiert sind, wenn sie auch in Wirklichkeit in manchen Fällen Stolonen keinen Ursprung geben, weil die allgemeinen physiologischen Bedingungen das Herauswachsen des Stolonen nicht erlauben.

9) Im Verlaufe des Versuchs wird die ursprüngliche Spezifizierung des aboralen Endes als Stolonen bildender Bezirk verändert und es wird zu einem Hydranten bildenden Bezirk. Dieser Anschauung entsprechend ist die Hydrantenbildung am aboralen Ende ein sekundärer Prozeß, der nichts mit der ursprünglichen Polarität zu tun hat, sondern durch den Wechsel der physiologischen Beschaffenheit angeregt wird, der im Verlauf des Versuchs eintritt. Daher repräsentiert die Verzögerung der Bildung des aboralen Hydranten die für die Veränderung der Spezifizierung nötige Zeit. Verschiedene Bedingungen, wie die Länge des Stückes, das Vorhandensein oder das Fehlen eines Hydranten am oralen Ende usw., können die Länge dieser Zeit wachsen oder abnehmen lassen.

10) Beobachtung und Versuch weisen darauf hin, daß die ursprüngliche Spezifizierung des aboralen Stückendes als Stolonen bildender Bezirk am größten ist und daß die damit verbundenen Reaktionen am intensivsten in den proximalen, am schwächsten in den distalen Stammbezirken sind, mit allen Abstufungen dazwischen. Die ursprüngliche Spezifizierung des oralen Endes als Hydranten bildender Bezirk folgt anscheinend der umgekehrten Regel. Wenn dies der Fall ist, besitzt *Tubularia* eine »echte Polarität«. Die ursprüngliche Polarität ist immerhin oft maskiert durch physiologische Veränderungen während des Versuchsablaufs.

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