THEORY OF REGENERATION BASED ON MASS RELATION.*

III. FURTHER EXPERIMENTS ON THE CAUSE OF THE POLAR CHARACTER OF REGENERATION.

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I.

It had been stated in a preceding paper that we have to decide between two possible explanations of the polar character of regeneration in the stem of Bryophyllum calycinum; namely, first, Sachs' idea that the polar character of regeneration in the stem is due to a difference in the chemical constitution of the ascending and descending sap, the ascending sap containing special shoot-forming substances collecting at the apex, the descending sap containing root-forming substances collecting at the base. The second possible explanation is that the anlagen for roots and shoots are contained in different histological elements of the stem and that the ascending sap reaches primarily the anlagen for shoot formation, while the descending sap reaches primarily the anlagen for root formation. This latter explanation does not exclude the possibility that there exist chemical differences between the ascending and descending sap; it only assumes that the chemical differences between ascending and descending sap which in all likelihood exist are not the cause of the polar character of regeneration in the stem.

* In the preceding papers the term mass action instead of mass relation was used. Since the objection might be raised that the proportionality between the mass of leaf or stem and the mass of shoots or roots regenerated by them may not be entirely the result of mass action, it is safer to use the term mass relation for the present instead of mass action.


463
We have already given one fact which seems to speak in favor of the second explanation and we will add some further observations favoring this explanation.

The sap in the leaf contains all the substances required for the growth of both roots and shoots. This is no mere surmise, but is demonstrated by the fact that from each notch of an isolated leaf both roots and shoots can arise, and that the mass of shoots and roots produced varies in proportion with the mass of the leaf. This leaves no doubt that the same tissue sap can give rise to entirely different organs and that the cause of the difference must lie in differences in the nature of the cells from which the different organs arise. It will be shown in this paper that the shoots or roots produced in a sufficiently small piece of stem containing one or more large leaves originate almost entirely from the sap sent out by the leaf. If it is intended to explain the polar character of the regeneration in this case on the basis of a chemical difference of the sap sent out by the leaf to the two opposite poles of the stem, it will be first necessary to explain how the sap sent out by a leaf can be separated into two chemically distinct masses, one to travel in an ascending the other in a descending direction in the stem. The writer is not aware that such an assumption is justified on the basis of our present knowledge.

Slender stems with five nodes (designated as 0, 1, 2, 3, and 4) were cut out from plants almost but not quite 1 year old. All the leaves were removed except the pair of leaves in the third node (Fig. 1). The stems were split longitudinally between the two leaves, and one leaf was left in connection with its half stem (I, Fig. 1), while the other leaf was detached and suspended with its apex dipping into water (III, Fig. 1). The two half stems, I and II, dipped also with their base into water. Half stem I, with a leaf attached in Node 2, produced larger shoots at the apex than the other half of the stem II with no leaf. The difference in root formation was in the same sense and still greater. The experiment lasted from October 18 to November 9. Six half stems without leaves (II, Fig. 1) produced 28 mg. dry weight of shoots, while the other six half stems each with a leaf produced (during the same time under equal conditions) 237 mg. dry weight of shoots. Of this quantity about 209 mg. were, therefore, furnished by the leaves. The six isolated sister leaves (III, Fig. 1) produced 292 mg. of shoots.
Fig. 1. Stems split longitudinally. Left half (I) with leaf in middle, produces a greater mass of shoots and roots than the right half (II) without a leaf. (October 18 to November 9, 1922.)
Hence more than two-thirds of the material available for shoot formation in the leaf was used for shoot formation in the apex of the stem. The six isolated leaves produced 102 mg. dry weight of roots while the six half stems with leaves attached produced 136 mg. Hence the total quantity of regeneration in the stems was 373 mg., while the total quantity of regeneration in the isolated leaves was 394 mg. This shows that the regeneration in the stems was determined chiefly by the sap sent out by the leaves; the leaves in connection with the stem produced neither roots nor shoots.

The pieces of half stem without leaf had not yet commenced to produce any roots. It may be well to use this fact to point out that the influence of the leaf on the root formation in the stem cannot be ascribed merely to water being sent by the leaf into the stem, since both stems with and without leaf dipped with their base into water in this experiment, so that the stems without leaves had all the water they needed for root formation at the base; yet their root formation was delayed and limited in comparison with a stem possessing a leaf. The leaf furnished, therefore, something more than water for the formation of the roots. The same reasoning holds for the influence of the leaf on the formation of shoots in the stem, since the stem dipping into water could absorb all the water needed for shoot formation.

The total results of the experiment are given in Table I.

<table>
<thead>
<tr>
<th></th>
<th>Dry weight of leaves</th>
<th>Dry weight of stems</th>
<th>Dry weight of regenerated shoots</th>
<th>Dry weight of regenerated roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Six half stems with leaves</td>
<td>1.993</td>
<td>1.889</td>
<td>0.237</td>
<td>0.136</td>
</tr>
<tr>
<td>II. Six half stems without leaves</td>
<td>1.836</td>
<td></td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>III. Six leaves</td>
<td>2.065</td>
<td></td>
<td>0.292</td>
<td>0.102</td>
</tr>
</tbody>
</table>

In this experiment the regeneration of roots was completely due to the material sent out by the leaf into the stem and over two-thirds of the dry weight of the shoots regenerated by the stem was also furnished by the sap from the leaf. Yet the character of the regeneration in the stem was as clearly polar as in the completely defoliated stems described in a preceding paper.
It was to be expected that the quantity of regeneration in an isolated piece of stem should vary with the mass of the leaf attached. In eight stems, of five nodes each, all the leaves except those in the third node were removed and the stems were split lengthwise. One leaf remained intact, while the sister leaf was reduced by cutting away about two-thirds of the leaf. The stems were suspended so that their base dipped into water. Fig. 2 shows that the half stems with reduced leaves produce a correspondingly smaller mass of apical shoots and roots. Table II gives the results.
If account is taken of the fact that part of the material sent by the leaf into the stem is consumed for the growth of the latter (as was shown in preceding papers), the mass of shoots produced in the stem varies approximately with the mass of the leaf.

In these experiments the base of the stem dipped into water and it might be argued that the substances taken by the stem from the water and carried in the ascending sap might have had a share in this result. For this reason the experiments were repeated on stems suspended entirely in moist air. The result was essentially the same.

<table>
<thead>
<tr>
<th>TABLE II.</th>
<th>Dry weight of leaves.</th>
<th>Dry weight of regenerated shoots.</th>
<th>Dry weight of regenerated roots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eight half stems with whole leaf</td>
<td>3.550</td>
<td>0.404</td>
<td>0.218</td>
</tr>
<tr>
<td>Eight half stems with reduced leaf</td>
<td>1.273</td>
<td>0.201</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Further facts supporting the idea that the polar character of regeneration in the stem of Bryophyllum is not due to the chemical differences in the ascending and descending sap can be given by experiments on the influence of gravity on root formation. It had been suggested in a preceding paper that we must distinguish in a plant between the sap which flows in regular vessels and the tissue sap which exists in the tissue spaces. This latter sap can follow the influence of gravity when the tissue spaces are sufficiently large; while the sap in the vessels cannot follow gravity at all or only to a negligible extent. We shall see a corroboration of this suggestion later in this paper. The fact that the tissue sap follows gravity gives us a chance to decide whether the polar character of regeneration in the stem is due to any chemical differences between ascending and descending sap or whether it is due to any difference in the nature of the tissues which these two kinds of sap reach primarily in the plant; the ascending sap reaching primarily the anlagen for shoots while the descending sap

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reaches primarily tissues which give rise to roots. We shall see that in a stem suspended horizontally the descending sap from a leaf collects in the lower side of such a stem giving rise to the formation of roots; and that this formation of roots in the under side of a stem increases with the mass of an apical leaf. If now the polar character of regeneration in a stem were due to a chemical difference between the ascending and descending sap from a leaf, such a root formation should not occur in the under side of the apical part of a stem which has a leaf at its base, since in this case the ascending sap should contain shoot-forming but no root-forming substances. We shall see, however, that a leaf at the base of a stem causes also root formation on the under side of the apical part of a stem placed horizontally and that this root formation is exclusively due to ascending sap sent out by the basal leaf. This leaves little doubt that the influence of a leaf on the polar character of the regeneration in a vertical stem of Bryophyllum is due not to any difference in the chemical constitution of the ascending and descending sap from a leaf, but is due to the fact that the descending sap reaches in a stem suspended vertically primarily anlagen for root formation while the ascending sap reaches primarily the anlagen for shoot formation.

When a completely defoliated stem is suspended horizontally in moist air, the polar character of regeneration is not changed as far as shoot formation is concerned. Shoots continue to form in the apex. The polar character of root formation undergoes, however, a change which becomes striking when not all the leaves are removed. It is true that roots continue to be formed at the extreme base of the stem and this root formation undergoes no change when the stem is put into a horizontal position. The change manifests itself in the fact, however, that in addition to this polar form of root formation there appears now a second form of root formation; namely, along the whole lower side of the stem. This latter effect is due to the collection of tissue sap on the lower side of the stem under the influence of gravity. This peculiar influence of gravity on the regeneration of horizontally placed stems finds its explanation in the assumption already referred to that there are two channels for the distribution of sap in the stem; first, by the vascular system, and second, by the gaps between cells and tissues. The sap flow in the vascular bundles is little if at all influenced by
gravity, and those forms of regeneration which depend upon the vascular sap are little if at all affected by gravity. We notice, therefore, that shoots continue to form in abundance at the apex and roots at the extreme base of a stem placed horizontally.

The tissue sap, however, follows gravity, collecting on the lower side of the stem where roots are formed but no shoots. In this respect a stem suspended horizontally differs from a leaf which when suspended horizontally forms both roots and shoots on the lower edges. This difference in the influence of gravity on regeneration in the leaf and the stem is a strong argument in favor of the tissue theory of polarity and against the hormone theory. For if it is true that the descending sap which is responsible for the root formation at the base of a stem has this effect because it reaches primarily root-forming tissue we should expect that the tissue sap collecting on the lower side of a horizontally placed stem should give rise only to roots but not to shoots, as it actually does.

Fig. 3. shows the regeneration which occurs in stems suspended horizontally in moist air. The upper stem is entirely defoliated. It has formed shoots at the apex and roots on the upper as well as on the lower side of the most basal, fifth node, but more on the lower side. There are also a few roots on the lower side in the fourth node and none on the upper. There is only a slight indication of the influence of gravity on the growth of roots. The geotropic curvature is only slight as it always is in completely defoliated stems.

The lower drawing in Fig. 3 gives the influence of a pair of apical leaves on the formation of roots in a stem which was suspended horizontally. Both the upper and lower stems in Fig. 3 were in the same aquarium and both experiments were carried out simultaneously (November 18 to December 13, 1923). At and near the base, the lower stem (with two leaves at the apex) forms roots on the upper side as well as on the lower. In Nodes 1, 2, and 3, however, and in the internode between 2 and 3, abundant roots are formed on the lower side exclusively. In order to prevent the stem from undergoing too much curvature and from being thrown altogether out of an approximately horizontal position, it was loosely tied to a piece of wood as shown in the drawing.
It can be shown that the mass of the roots formed in horizontally placed stems increases with the mass of the apical leaf. The two stems in Fig. 4 were suspended simultaneously, the lower stem with a whole leaf at the apex formed more roots than the upper stem with a leaf reduced in size (May 4 to June 5, 1923).

![Diagram of a stem with roots forming on the underside](attachment:stem_with_roots.png)

**Fig. 3.** Stems suspended horizontally form roots on the under side of the stem. Lower stem with two apical leaves forms more roots than upper stem without leaves. (November 18 to December 13, 1923.)

It had already been shown in a preceding paper that the mass of air roots formed in a stem suspended horizontally in moist air increases in proportion with the mass of the apical leaf, so that there is no doubt about the fact that the descending sap sent out by a leaf is responsible for the greater part of roots formed in a stem placed horizontally.
Now if the sap sent out by the apical leaf in the ascending direction is as capable of causing root formation as is the descending sap, we should be able to demonstrate that when a stem possessing a pair of leaves at the base is suspended horizontally, roots will also form on the lower side of the stem apically from the leaf. That this is the case is shown in Fig. 5. The stem had a pair of large leaves at the base, one of which dipped into water. In order to prevent excessive geotropic curvature the stem was loosely fastened to a piece of wood. Nevertheless some geotropic curvature occurred. The stem has formed no
roots on the upper side but an abundant mass of roots on the lower side and some on the sides. The drawing was made on the 11th day of the experiment. Since the same stem without a leaf would in this time have formed practically no roots on the lower side the enormous root formation on the lower side of the apical stem must have been due...
to the ascending sap sent out by the basal leaves. The same results were obtained in a large number of experiments though the root formation varies in different stems, especially with the mass of the leaf. But the same is true for the root formation caused by the apical leaf.

Fig. 6. See legend of Fig. 5. (December 16, 1923 to January 10, 1924.)

On account of the seeming importance of the experiment for the theory of polarity in regeneration, a few more results of a similar character may be described. Fig. 6 is a stem which was straight at the beginning and had been suspended horizontally. The stem had a
pair of large leaves at the base. Roots were formed on the lower side of the stem only, not only basally from the leaves but also apically. It is worthy of notice that some of the roots situated apically from the leaf developed in the internode. The experiment lasted from December 16, 1923 to January 10, 1924.

Fig. 7 is a similar experiment where the stem had only one leaf at the base. The experiment lasted from December 17, 1923 to January 10, 1924.

While a stem without a leaf, when suspended horizontally in moist air, forms few roots, a considerable number of roots is formed in such a stem when it contains one or two leaves at the base. The root formation must in these cases, therefore, be due to material sent out by the leaf in the ascending sap.

It follows from this that the ascending sap sent out by a leaf gives rise to roots if it reaches cells capable of forming roots. If, therefore, a stem suspended vertically gives rise only to shoots it must be due to the fact that in a stem suspended vertically the ascending sap does not collect primarily in the cells where roots can be formed, but collects primarily near the most apical anlagen for shoots.
In all these cases the stems in which the roots formed had bent geotropically the under sides of the stem where the roots grew being convex. It might be argued that this convex form was the cause of the root formation. This is refuted by the fact already published years ago, that if a stem is bent passively and suspended so that the convex side of the stem is above, the roots form on the lower concave side of the stem.\textsuperscript{8}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig8.png}
\caption{Leaf in middle of stem. Root formation and geotropic curvature basally from the leaf. (December 23, 1923 to January 10, 1924.)}
\end{figure}

When the leaf is in the middle of a stem originally suspended horizontally the roots form chiefly in that part of the stem which is situated basally from the leaf, for the reason that only this part remains in a sufficient horizontal position, the part of the stem situated

\textsuperscript{8} Loeb, J., \textit{Bot. Gaz.}, 1917, lxiii, 25.
apically from the leaf assuming a vertical position on account of the geotropic curvature of the stem (Fig. 8).

A few words may be said in regard to the geotropic curvature. When a piece of stem suspended horizontally has one or two leaves at the base (as in Figs. 5, 6, and 7), geotropic curvature takes place in the stem apically from the leaf; when the leaf is in the middle chiefly basally from the leaf. This statement must replace the statement made in a preceding paper that the piece of stem apically from the leaf cannot bend geotropically; which is true only when the leaf is in the middle of the stem.

SUMMARY AND CONCLUSION.

1. Small stems with one large leaf in the middle produce shoots and roots chiefly at the expense of material sent into the stem by the leaf. Since the sap of the leaf can cause the formation of roots and shoots in the same notch of the leaf, it is difficult to assume that the polar character of the regeneration in the stem is due to a chemical difference in the ascending and descending sap sent by the leaf into the stem.

2. When the ascending sap sent by a leaf into the stem is caused with the aid of gravity to reach those tissues of the stem from which roots are formed, an abundant root formation is produced by the ascending sap.

3. These two observations in connection with a fact already published in a preceding paper support the idea that the polar character of regeneration in a stem of Bryophyllum is not due to any of the chemical differences between the ascending and descending sap but to a difference in the nature of the tissues or anlagen which are primarily reached by the ascending and descending sap.