THEORY OF REGENERATION BASED ON MASS ACTION. II.

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I.

The polarity in the regeneration of an isolated piece of stem of *Bryophyllum calycinum* expresses itself by two characteristics which must be treated separately; namely, first, the fact that regeneration occurs only at the extreme ends, and, second, that the character of the regenerated organs is different at the opposite ends, shoots being formed at the most apical node and roots at the extreme basal end of the piece.¹ The second fact had been explained by Sachs on the assumption that the descending sap sent out by the leaf contains specific substances fit for root formation while the ascending sap sent out by the leaf contains specific substances for shoot formation. This explanation does not seem tenable any longer in view of the fact, first, that the sap of the leaf favors root and shoot formation in the same notch of a leaf, and second, that it can be shown that under proper conditions the descending sap favors also shoot formation in the stem.² The difference in the character of the regenerated organs at the opposite ends of a piece of stem must therefore have a different reason and it was suggested that the descending sap reaches primarily cells or tissues which can give rise to roots and not to shoots, while the ascending sap primarily reaches tissues which can give rise to shoots.¹ Differences in the chemical nature of the descending and ascending sap sent out by the leaf may or may not exist; if they exist they cannot be the cause of the different character of the organs formed at the opposite ends of the stem.

If by mutilation the descending sap from a leaf can be deflected from its path, it may give rise to shoots as was shown in the preceding paper.

That the regeneration in a piece of isolated stem of _Bryophyllum_ is confined to the extreme ends of a piece of stem is, however, only a secondary phenomenon which demands an entirely different explanation than the fact that the character of the two regenerates is different. In a preceding paper it had already been shown that in the beginning, _i.e._, during the first 8 or 10 days, at greenhouse temperature, the regeneration in a piece of stem does not possess that pronounced character, which is understood by polarity; namely, that roots are only formed at the extreme base and shoots only at the extreme apex. One of the writer's earlier experiments\(^a\) may be mentioned to illustrate this fact.

Pieces of stem of _Bryophyllum calycinum_ are cut out from vigorous plants and all leaves with the exception of the two leaves at the most basal node are removed from the pieces. The base of the stems below the leaves dips into water. In this case, air roots grow out from the nodes above the leaves before they grow from the base of the stem; and shoot formation commences not only in the most apical buds but also in the buds in the second or third node below the apex. Under these conditions there is almost no indication that the character of regeneration in the stem is polar.\(^a\)

At greenhouse temperature this may all be observed within the first 8 or 10 days of the experiment, then the picture changes. At the base of the stems (dipping into water) roots now commence to appear and they grow more rapidly than the air roots which had appeared previously in the nodes of the stem. The air roots in the more apical nodes now begin to wilt and soon disappear. The more rapid growth of the basal roots dipping into water seems to suppress the further growth of the air roots which had previously formed. Likewise the shoots in the most apical node now commence to grow more rapidly than the anlagen for shoots in the nodes below the apex and the growth of these latter shoots now stops also. That feature of polarity which consists in the restriction of root formation to the extreme base and of shoot formation to the extreme apex of the piece of stem is not

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\(^a\) Loeb. _J. Gen. Physiol._, 1918-19, i, 687.
a primary but a secondary phenomenon. We will show that this same phenomenon can be demonstrated in a leaf and that it can be shown in this case that it is the expression of the following general rule in regeneration which the writer had already expressed in earlier publications; namely, that when in an isolated leaf (or in a piece of stem) the rate of growth of one type of organs is accelerated, all the sap will flow to these more rapidly growing organs, with the result that the growth of the competing organs will be suppressed. It is intended to furnish the quantitative proof for this rule.

![Diagram of leaf regeneration](image)

**Fig. 1.** Difference in the place of regeneration when the leaf is suspended entirely in air and when its apex dips into water.

When a leaf of *Bryophyllum* is suspended in moist air, shoots and roots will grow from the notches in the middle of the leaf or nearer the petiole where the leaf is more fleshy and where more sap is available than at the thin apex of the leaf (see Fig. 1, leaf marked “in air”). When, however, the apex of the leaf is dipped into water, while the rest is in air (Fig. 1, leaf marked “in water”), roots and shoots will grow out only from those notches of the apex which dip into the water.
or are close to the surface of the water. The other notches commence also to grow at first and tiny roots and tiny shoots may be formed; but as soon as the notches which dip into water commence to grow, all the growth of the shoots and roots in the other notches not in contact or in close proximity with water will cease. It can be shown that the reason for this inhibition is that the notches dipping into water grow more rapidly than the notches growing in air and that as a consequence of this more rapid growth a flow of the sap of the whole leaf towards the more rapidly growing notches is established. Thirteen pairs of sister leaves were used for the experiment, one leaf of each pair dipping with its apex into water while the sister leaf was suspended entirely in air. Table I shows that the mass of shoots and roots produced in the leaves dipping into water was greater than the mass of shoots and roots produced simultaneously and under equal conditions by the sister leaves suspended in air.

<table>
<thead>
<tr>
<th>TABLE I.</th>
<th>13 leaves dipping in water</th>
<th>13 leaves suspended in air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight of leaves (gm.)</td>
<td>1.943</td>
<td>1.909</td>
</tr>
<tr>
<td>Dry weight of shoots (gm.)</td>
<td>0.524</td>
<td>0.322</td>
</tr>
<tr>
<td>Dry weight of roots (gm.)</td>
<td>0.123</td>
<td>0.051</td>
</tr>
<tr>
<td>1 gm. dry weight of leaves produced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots (mg.)</td>
<td>270</td>
<td>63</td>
</tr>
<tr>
<td>Roots (mg.)</td>
<td>169</td>
<td>27</td>
</tr>
</tbody>
</table>

It is obvious that the leaves suspended in air form a much smaller quantity of dry weight of shoots and roots per gm. of dry weight of leaf during the same time and under the same conditions than the leaves dipping into water. Hence, if we accelerate the growth of some notches in the leaf, e.g., by dipping them into water, we thereby inhibit the growth in the other notches. That this inhibition is merely due to the fact that all the material available in the leaf now goes to the shoots in the apex can also be proven by quantitative experiments. The writer had shown in a previous publication that if an isolated leaf is suspended vertically and sidewise in moist air (Fig. 2), i.e., the middle rib being in a horizontal position, while the surface of the leaf is vertical (Fig. 2), shoots and roots develop with preference, and in most cases exclusively, on
the lower edge of the leaf. This can be explained on the assumption that through the influence of gravity more liquid collects in the lower edge and that as a consequence the growth in these lower notches is accelerated, and as a further consequence a flow of material to the more rapidly growing lower notches is established, whereby all the material available in the leaf flows to the lower edge.

A number of pairs of sister leaves were suspended vertically and sidewise in air, one leaf of each pair remaining intact, while the upper half of the sister leaf was cut away (as shown in Fig. 2). The whole leaves formed practically twice as great a mass of shoots and roots on the lower edge as the half leaves and this difference is noticeable in Fig. 2. Thirteen whole leaves and their thirteen half sister leaves were chosen for comparison in Table II.

This proves that the excess regeneration in the whole leaves was produced by material furnished by both the lower and the upper half
of the leaves, regeneration in the upper half being as a consequence impossible, since almost all the material available for regeneration in the upper half was consumed for regeneration in the lower half.

In the case just discussed the regeneration in the lower half of the leaf is accelerated since owing to the action of gravity the liquid in the leaf collects in the lower half. This happens only when the leaf is suspended sidewise in the air but not when it is suspended in the air with the apex down; as is shown by a comparison of the left-hand drawing in Fig. 2 with the right-hand drawing in Fig. 1. The reason for this difference is probably that the apex of the leaf is very thin in comparison with the fleshy middle part of the leaf so that under the influence of gravity liquid cannot collect as abundantly in the apex of the leaf as in the lateral parts.

II.

It had been shown in a preceding paper¹ that the mass law holds also for the regeneration of roots and shoots in a defoliated piece of stem of Bryophyllum when exposed to light. The dry weight of shoots and roots regenerated by such pieces in a given time under given conditions is approximately in proportion to the dry weight of the stem; and when a long stem of e.g. eight nodes is cut into eight pieces, each containing only one node, the total dry weight of the sixteen shoots produced by these pieces in a given time equals approximately the dry weight of the two apical shoots produced in the same time and under the same conditions by a stem of equal dry weight, not cut into smaller pieces. This shows that all the material available for regeneration in a larger piece of defoliated stem goes into the apical shoots and basal roots.

When a piece of stem is cut into as many pieces as there are nodes the shoots commence to grow out from the nodes regardless of the order in which they had originally been arranged in the stem; but in each piece the rate of growth corresponds approximately to the dry weight of the piece of stem, except in very old pieces of stem where not all material may be alive or in the very young pieces at the apex, where the axillary buds capable of growing into shoots may not yet have been properly developed.¹
In the introduction to this note attention has been called to the fact that when regeneration begins in a piece of stem under the conditions described, regeneration is at first not markedly polar, since the first roots do not grow out at the base of the stem, but from the more apical nodes; and the anlagen for shoots do not grow out only in the apical node, but also below. The polar character becomes only established after about a week, when the roots at the extreme base begin to appear, and when the rate of growth of the most apical shoots suddenly exceeds that of the anlagen for the buds in the lower nodes. On the basis of the quantitative experiments on leaves described in this paper we must conclude that the more rapid growth of the most apical bud and of the basal roots which starts after about a week coincides with a collection of sap at the extreme ends of the piece of stem. This collection of sap caused by the block of the ascending sap at the apical node and of the descending sap at the base of the stem acts in a similar way as the collection of sap on the lower edge of a leaf suspended sidewise; namely, accelerating growth at the place where the sap collects. This acceleration of growth now influences the sap flow so that all the ascending sap goes to the apical node, and all the descending sap to the base, cutting off that supply of material for the rest of the stem which was available immediately after the operation.

SUMMARY.

1. Quantitative proof is furnished that all the material available for shoot and root formation in an isolated leaf of Bryophyllum calycinum flows to those notches where through the influence of gravity or by a more abundant supply of water growth is accelerated. As soon as the acceleration of growth in these notches commences, the growth of shoots and roots in the other notches which may already have started ceases.

2. It had been shown in a preceding paper that the regeneration of an isolated piece of stem may be and frequently is in the beginning not markedly polar, but that after some time the growth of all the roots except those at the base and of all the shoots except those at the apex is suppressed. This analogy with the behavior of regenera-
tion in a leaf in which the growth in one set of notches is accelerated, suggests that in an isolated stem a more rapid growth is favored at the extreme ends (probably by a block of the sap flow at the extreme ends) and that when this happens the total flow of ascending sap goes to the most apical buds and the total flow of the descending sap goes to the most basal roots. As soon as this occurs, the growth of the other roots and shoots is suppressed.