QUANTITATIVE LAWS IN REGENERATION.

III. The Quantitative Basis of Polarity in Regeneration.

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I. INTRODUCTION.

It has been shown in preceding papers that the dry weight of shoots and roots produced under equal conditions of illumination, moisture, and temperature in sister leaves of *Bryophyllum calycinum* varies approximately in direct proportion with the dry weight of the leaves; and that the same is true for the mass of shoots produced in small pieces of stem connected with a leaf.[1] It had been known that when a piece of stem is left in connection with a leaf, the mass of shoots produced by the leaf is less than when the leaf is completely isolated, and the writer had been able to show that in this case the stem connected with the leaf gains approximately as much in dry weight as the dry weight of the shoots and roots in the leaf would have been if the leaf had been completely isolated from the stem.[2] The inhibitory influence of the stem on the shoot and root formation in the leaf was in this case due to the fact that when the leaf is connected with a stem, that part of the material which could have been utilized for the formation of new shoots and roots in the leaf now goes into the stem. It is intended to show in this paper that the same simple quantitative relations suffice to account for the polar character of regeneration in a defoliated stem of *Bryophyllum*.[3]

The reader will remember that each node of the stem of this plant has two dormant buds capable of growing into shoots. When a piece of defoliated stem is cut from a plant and suspended in moist air, only

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[1] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 81; 1919–20, ii, 297, 651. *Science*, 1917, xlv, 436. *Bot. Gaz.*, 1918, xlv, 150. *Ann. Inst. Pasteur*, 1918, xxxii, 1.

[2] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 297, 651.

[3] Loeb, J., *Science*, 1921, liv, 521.

the two buds of the most apical node will grow into shoots, while the buds in all the nodes below will remain dormant. Permanent roots will grow only at the base of each piece, though transitorily air roots may begin to form in any node, but these will dry out as soon as the basal roots are growing.[4] Fig. 1 illustrates this polar character of regeneration in defoliated pieces of stem suspended in moist air. When, however, a long defoliated stem is cut into as many

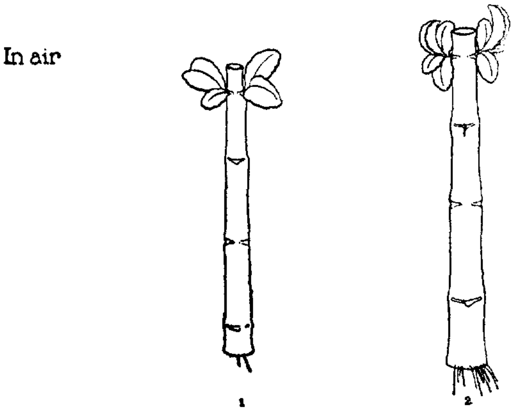

FIG. 1. Pieces of stem from the same plant, (1) apical, (2) basal. Suspended in moist air, shoots formed only in the apical node, roots at the base. Mass of shoots and roots is larger in the basal piece (2) which has the larger mass. Duration of experiment October 4 to November 7, 1921.

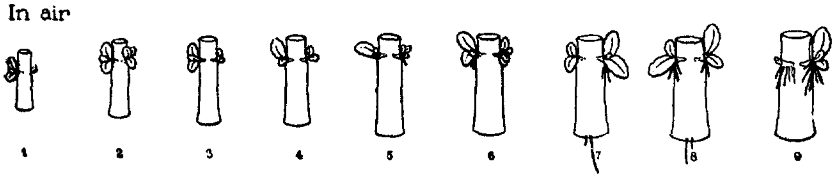

FIG. 2. Stem cut into small pieces with 1 node each. Suspended in same aquarium and simultaneously with large stems in Fig. 1. (1) was the most apical, (9) the most basal piece, the serial number denoting the original position of the pieces in the plant. Each piece of stem forms 2 shoots in its node, but the relative mass of the shoots varies with the relative mass of the stem, not with the serial number of the node.

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[4] Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 687.

pieces as there are nodes, then all the dormant shoot buds of the stem will grow out into shoots (Fig. 2). The stems in Figs. 1 and 2 were cut out at the same time and suspended in moist air in the same vessel.

The results remain about the same when the basal ends of the pieces are dipped into water, the only difference being that often not only the two buds in the most apical node of a long piece of stem grow out but also one or two buds of the node below (Fig. 3). The amount of growth of shoots and roots is also greater in the stems put with the base in water (Fig. 3) than when the stems are suspended in moist air (Fig. 1). When pieces of stem with only one node each are put into water, each piece forms shoots at its node (Fig. 4). The

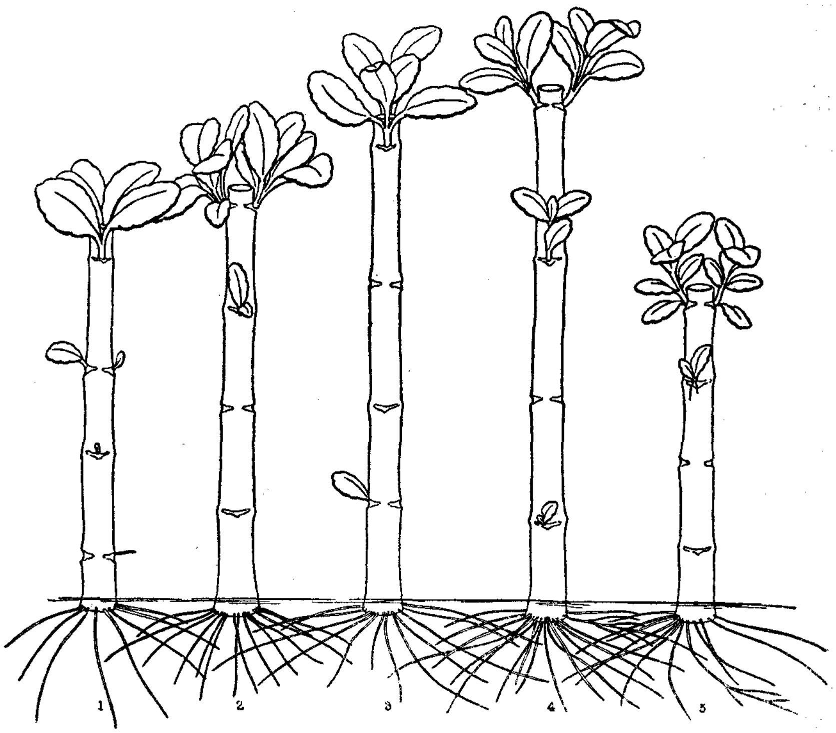

FIG. 3. Same experiments as Fig. 1, only that the long pieces of stem were put with their bases into water. Duration of experiment from September 27 to October 22, 1921. All stems were cut from one plant.

question is, Why do only the most apical buds of a long defoliated stem grow out? Bonnet had suggested that the ascending sap of a plant was shoot-producing and the descending sap was root-producing. Sachs pointed out that when a piece of stem was cut out from a plant the ascending sap was blocked at the apex and that hence the shoot-producing substances must collect at that end of the stem giving rise to shoots at the apical node; while the descending sap is blocked at the base, giving rise to root formation at that end.

The problem then exists to prove or disprove the old suggestion of Bonnet and Sachs. The formation of shoots or roots is a synthetic process or a series of catenated synthetic processes, in which soluble materials, such as sugars, amino-acids, and other substances, are synthetized into the larger molecules of proteins, compounds of the cellulose type, and others. If the theory of Bonnet and Sachs is correct, it must be possible to show that the two shoots formed at the apex of a long defoliated piece of stem have, within the limits of the accuracy of the experiments, approximately the same dry weight as the dry weight of all the shoots would have amounted to if the stem had been cut into as many small pieces as it contained nodes.

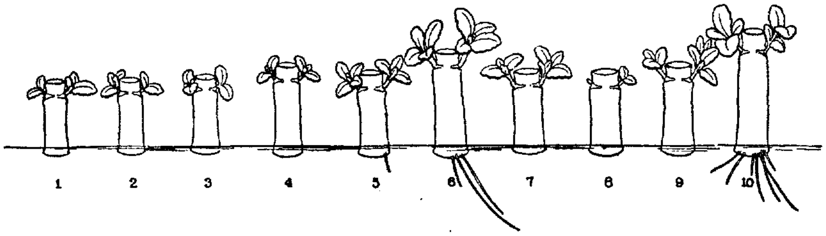

FIG. 4. Stem of one plant cut into ten small pieces, the serial number indicating their position in the plant, (1) being the most apical, (10), the most basal piece. Base in water. Experiment simultaneous with Experiment III. Each piece has formed 2 shoots the relative size of which does not follow the serial number of the stem, but the relative size. The size of each shoot of the pieces is much smaller than the size of the shoots formed simultaneously by the larger stems in Fig. 3. The latter stems all have roots, while only the two largest pieces of stems (6) and (10) in Fig. 4 have formed roots.

By comparing the amount of shoots formed in the one-node pieces in Fig. 2 or 4 with those of the four-node pieces in Fig. 1 or 3, the reader will notice that the shoots are greater in the larger pieces of stem, and the same fact is obvious from all the other figures in this paper. It is almost obvious from a glance at the figures that the mass of shoots formed increases with the mass of the stem. If the mass of shoots produced at the apex of large pieces of stem is approximately equal to the mass of shoots which the same stems would have produced had they been cut into as many pieces as the stems contained nodes (*i.e.*, into one-node pieces), it will be necessary to show that within the limits of the experimental errors, the mass of dry weight of shoots produced per gram of dry weight of stem is about the same regardless of whether the stems are long or whether they are subdivided into one-node pieces.

This was tested in various ways. The defoliated stem of a very large plant was cut into 5 pieces, each possessing 4 nodes (Fig. 3), and the defoliated stem of a second plant was cut into 10 small pieces of 1 node each (Fig. 4). The pieces dipped with the base into water and the large and small pieces were suspended in the same aquarium. The experiment lasted from September 27 to October 22, 1922. The shoots were then cut off and both shoots and stems were dried for 24 hours in an oven at about 100°C. The result was as follows: The dry weight of the 5 large stems (Fig. 3) was 13.670 gm., and the dry weight of their 16 shoots was 0.495 gm. The shoot production was therefore 36 mg. per gram of stem (all measured in dry weight). The dry weight of the 10 short pieces of stem with 1 node each (Fig. 4) was 2.880 gm., and the dry weight of 19 shoots was 0.115 gm., or 1 gm. of dry weight of stem produced 40 mg. of dry weight of leaves. These two figures, 40 mg. and 36 mg., agree sufficiently closely to show that under equal conditions the production of shoots of defoliated pieces of stem occurs in proportion with the mass of the piece of (defoliated) stem; or, in other words, the mass of shoots produced at the apex of the large defoliated stems of Fig. 3 is approximately equal to the mass of shoots the same stems would have produced if all the dormant buds of each stem had been able to grow out.

The experiment in Figs. 1 and 2 gave a similar result. The experiment lasted from October 4 to November 7. 5 large stems with 4 nodes each (Fig. 1) having a dry weight of 5.486 gm. produced 10 shoots with a dry weight of 0.114 gm.; *i.e.*, 20.8 mg. of shoot per gram of stem.

4 short pieces of stem with 2 nodes each, having a dry weight of 3.214 gm., produced 8 shoots with a dry weight of 0.0668 gm.; *i.e.*, 20.7 mg. of shoot per gram of stem.

A third stem was cut into 9 pieces with 1 node each (Fig. 2) possessing a dry weight of 3.270 gm., giving rise to 17 shoots with a dry weight of 0.050 gm.; *i.e.*, 15.3 mg. of shoot per gram of stem.

The first two figures are identical, the last figure is a little low. In these experiments the end of the piece may suffer (by drying out or falling a prey to fungi) and this creates an error which is especially noticeable when a stem is cut into many small pieces. But in spite of these sources of error the results are remarkably clear and consistent.

It seemed of interest to compare the behavior of defoliated stems split longitudinally. In this case the two halves should give approximately equal results.

II. Experiments with Split Stems.

Experiments were made with stems split longitudinally as indicated in Fig. 5. Only pieces from the middle of the stem of a large plant were used, for reasons to be given later. Stems with 4 nodes each, were split longitudinally and one half was cut transversely into 2 pieces with 2 nodes each, *a'*, *b'*, and *c'*, *d'*, respectively (see Fig. 5). The other half with the 4 nodes *a*, *b*, *c*, and *d* was not cut transversely. All 3 pieces (Fig. 5) were put with their bases into water. It was to be expected that the sum of the dry weight of the shoots produced by the 2 small pieces with 2 nodes each should equal the dry weight of the shoots produced by the larger pieces with 4 nodes each. Fig. 5 shows at a glance that this is approximately the case and the dry weight determinations confirm this.

The first experiment was carried out on 7 stems, a second experiment on 16 stems. Table I gives the result.

It is therefore obvious that the dry weight of the sum of the shoots produced by the small pieces *a'*, *b'*, and *c'*, *d'*, approximately equals the dry weight of the shoots produced by the big pieces, *a*, *b*, *c*, and *d* (Fig. 5), or, in other words, the mass of shoot produced at the apex of the large pieces is approximately equal to the dry weight of the shoots the same stems would have produced if the buds of every second node had been able to grow.

TABLE I.

Experiment No.	Duration of experiment.	Number of pieces.	Dry weight of shoots produced.	Dry weight of stems.	Shoots produced per gram of stem.
	1921		*gm.*	*gm.*	*mg.*
I	Nov. 3–Dec. 6.	7 four-node pieces, *a*, *b*, *c*, *d*.	0.1545	4.290	36.0
		14 small pieces, *a′*, *b′*, *c′*, *d′*.	0.147	3.822	38.7
	1921-22				
II	Dec. 8–Jan. 10.	16 four-node pieces, *a*, *b*, *c*, *d*.	0.750	16.646	45.0
		32 two-node pieces, *a′*, *b′*, *c′*, *d′*.	0.577	14.527	39.5

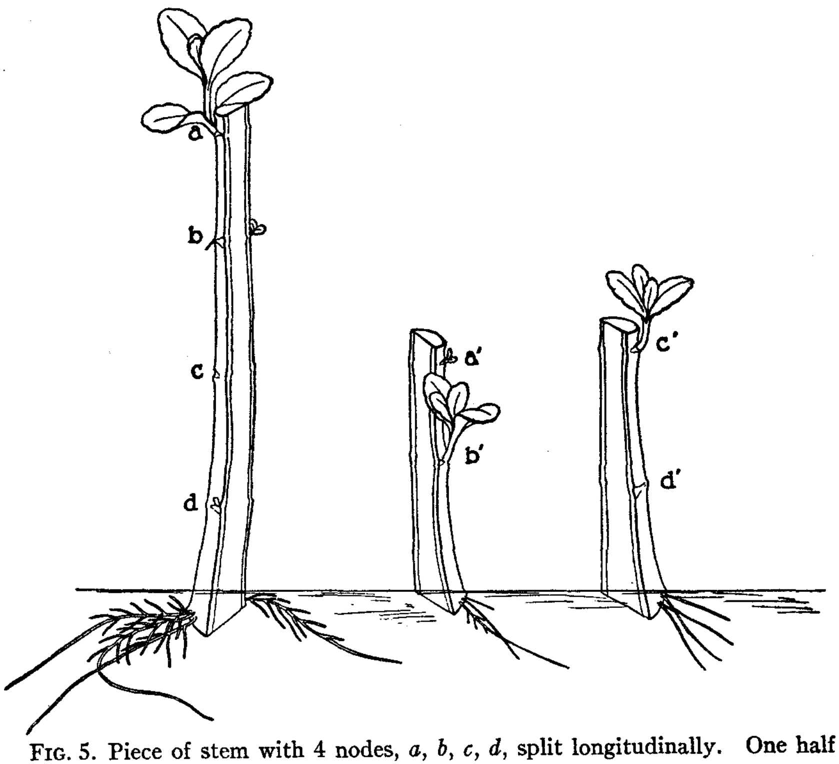

FIG. 5. Piece of stem with 4 nodes, *a*, *b*, *c*, *d*, split longitudinally. One half cut transversely into two pieces, *a′*, *b′*, and *c′*, *d′*. The half *a*, *b*, *c*, *d*, produces 1 shoot which about equals in mass the 2 shoots produced by *a′*, *b′*, and *c′*, *d′*. Duration of experiment December 9, 1921, to January 4, 1922.

III. Small and Large Pieces of the Same Stem.

A third series of experiments was as follows: Long pieces of stem, containing about 10 nodes, were cut out from the same plant more than 1 year old (Fig. 6). The middle piece of about 6 nodes (piece 3 in Fig. 6) served for the experiment, two small pieces, 1 and 2, containing 1 node each and situated apically, and 2 small pieces, 4 and 5, also containing 1 node each, situated basally from the large middle

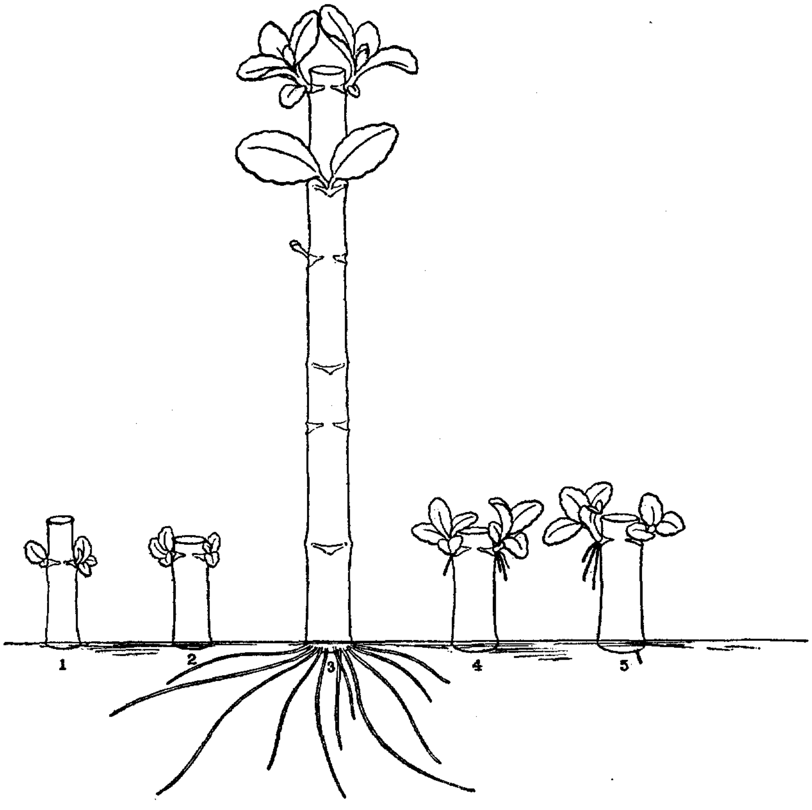

FIG. 6. Five pieces cut from the stem of the same plant, (1) and (2) apical pieces with 1 node each, (3) middle piece with 6 nodes, and (4) and (5) the basal pieces with 1 node each. The large middle piece produces larger shoots than either the more apical or more basal small pieces. The large middle piece has ample roots while only the longer basal piece commences to form a root. Duration of experiment October 25 to November 21, 1921.

piece in the same stem, serving as controls. In other experiments of the same character, pieces containing about 14 nodes were cut out from the stem of the same plant; 2 small pieces at the apex, each containing 2 nodes (1 and 2, Fig. 7), and 2 small pieces at the base each

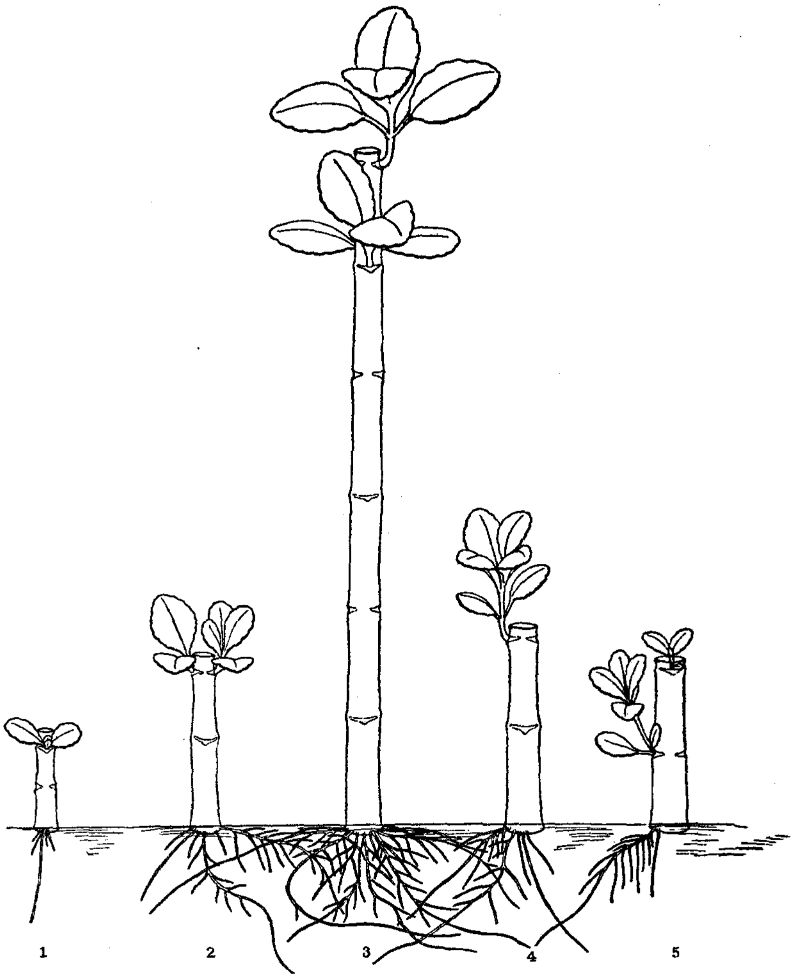

FIG. 7. Similar experiment as Fig. 6, except that the small pieces have 2 nodes each. Shoots and roots are formed in proportion to mass of stem. Duration of experiment November 16 to December 19, 1921.

containing also 2 nodes (4 and 5, Fig. 7), were used as controls, while the middle piece (3, Fig. 7) served for the main experiment. All the pieces dipped with their bases into water.

It is obvious from Fig. 6 that the large pieces of stem (3) produced larger masses of shoots than the small pieces 1 and 2 or 4 and 5 during the same time and under equal conditions. It may also be pointed out that these large middle pieces (3) formed their basal roots earlier than the small pieces (Fig. 6), and that the mass of their roots remained greater than the mass of roots in the small pieces (Fig. 7).

It turned out that the shoot production in the most apical pieces of stems 1 and 2 was usually irregular, as a rule too small, so that these pieces were not well usable as controls. The basal pieces, 4 and 5, however, behaved normally. It seems that this abnormal behavior of the small apical pieces is found as long as the leaves connected with this piece are still small and growing. It is therefore well to use in these experiments that part of the stem which is naturally defoliated or the leaves of which are about to fall. It may also be well not to use pieces of stem too near the roots. After 3 to 5 weeks the dry weight of the shoots and of the stem used in these experiments were determined. Since some of the small pieces of stem fall often a victim to fungi only one of the 2 small pieces, apical or basal, was used as a control.

Experiment I. October 25, 1921, to November 25, 1921.

		Dry weight of shoots per gram of stem.
6 long pieces with 6 nodes each.	*gm.*	*mg.*
Dry weight of stems	9.260	
" " " 13 shoots	0.260	28.0
" " " roots	0.057	
Control a. 7 short basal pieces with 1 node each.		
Dry weight of stems	2.895	
" " " 13 shoots	0.088	30.4
" " " roots	0.003	
Control b. 12 short apical pieces with 1 node each.		
Dry weight of stems	1.428	
" " " 18 shoots	0.0236	16.5

It is obvious that the apical control pieces gave too small a production of shoots (16.5 mg. per gram of stem), while the basal control pieces produced approximately the same amount of shoots per gram of stem, namely 30.4 mg. as compared with 28.0 for the large pieces.

Experiment II. November 2, 1921, to December 6, 1921.

		Dry weight of shoots per gram of stem.
5 long pieces of stem with 6 nodes each.	*gm.*	*mg.*
Dry weight of stems	6.486	
“ “ “ 10 shoots	0.272	42.0
“ “ “ roots	0.0458	
Control a. 4 short basal pieces with 1 node each.		
Dry weight of stems	1.058	
“ “ “ 8 shoots	0.041	39.0
“ “ “ roots	0.0034	
Control b. 5 short apical pieces with 1 node each.		
Dry weight of stems	0.544	
“ “ “ 10 shoots	0.018	33.0

Again the short basal control pieces produce about as much shoot material per gram (39 mg.), as the large pieces (42 mg.), while the apical controls produce less, namely, 33 mg. We will omit the apical controls in the further tabulation of experiments on account of the irregularity of the results.

Experiment III. November 16, 1921, to December 20, 1921.

		Dry weight of shoots per gram of stem.
9 long pieces of stem with 6 nodes each.	*gm.*	*mg.*
Dry weight of stems	18.658	
“ “ “ 26 shoots	0.944	50.3
“ “ “ roots	0.1428	
Control. 18 small basal pieces of 2 nodes each.		
Dry weight of stems	18.147	
“ “ “ 36 shoots	0.800	44.0
“ “ “ roots	0.136	

Experiment IV. October 22, 1921, to November 15, 1921.

4 long pieces of stem with 4 nodes each.		
Dry weight of stems	4.214	21.0
“ “ “ 8 shoots	0.089	
Control. 4 short basal pieces of 2 nodes each.		
Dry weight of stems	2.492	19.0
“ “ “ 8 shoots	0.0475	

Experiment V. October 11, 1921, to November 1, 1921.

		Dry weight of shoots per gram of stem.
4 long apical stems with 6 nodes each.	gm.	mg.
Dry weight of stems	3.921	
" " " 8 shoots	0.113	29.0
" " " roots	0.0134	
Control. 4 basal pieces of 2 nodes each.		
Dry weight of stems	3.744	24.0
" " " 10 shoots	0.090	

Experiment VI. December 11, 1921, to January 17, 1922.

7 long apical stems with 6 nodes each.		
Dry weight of stems	6.634	
" " " 12 shoots	0.340	51.0
" " " roots	0.0512	
Control. 7 short basal pieces of 2 nodes each.		
Dry weight of stems	3.560	
" " " 12 shoots	0.1770	49.6
" " " roots	0.0128	

If we consider only those figures in the experiments where the small control pieces of stem were situated basally from the long stem (the pieces 4 and 5 in Figs. 6 and 7), we notice that the differences of shoots produced per gram of dry weight of the controls differ never more than 25 per cent from those produced by the large pieces of stem and that in some cases the difference is only about 6 per cent. Considering the limitations in the experimental conditions—especially the fact that part of the stem may not function normally, especially the ends near the cut, or the fact that individual buds may have been injured by parasites, etc.—the agreement of the figures seems remarkable.

These results leave no doubt that within the limits of accuracy of these experiments the dry weight of the shoots produced at the apex of a long piece of defoliated stem is about equal the mass of shoots the same stem would have produced had the buds in all of its nodes been able to develop.

IV. *Regeneration of Roots.*

Two kinds of roots are formed in an isolated piece of stem, suspended in moist air and dipping with the base in water, first, air roots in the nodes, and later roots at the basal end of the stem regardless of the node (Figs. 1, 3, and 7). The air roots in the nodes grow out sooner than the basal roots but as soon as the basal roots grow out the air roots dry out and die. This has been discussed in a previous paper. We are interested here only in the basal roots since they alone are connected with the problem of polarity. The regeneration of the basal roots differs from the formation of apical shoots in this, that the apical shoots begin to grow out almost immediately after the defoliated piece of stem is isolated, while there is a long latent period before the basal roots make their appearance. For this reason quantitative measurements correlating the mass of the basal root formation with the mass of stem require probably a longer time than that in our experiments. A glance at the drawings will, however, convince the reader that the root formation commences sooner in the stems with larger mass than in the stems with smaller mass, regardless of the original position of the piece of stem in the plant.

Thus in Fig. 6 the large middle piece (3) forms roots before either of the 2 more basal pieces form roots, and Fig. 7 shows that the relative mass of roots produced seems also to run parallel with the relative mass of the piece. The same phenomenon is shown if we compare Fig. 1 with Fig. 2, or Fig. 3 with Fig. 4. It is also obvious in Fig. 5, so that we can say that the mass of roots produced by pieces of defoliated stem of *Bryophyllum calycinum* increases under equal conditions with the mass of the stem.

V. *Influence of Light on Regeneration in a Defoliated Stem.*

8 long defoliated stems were suspended into an aquarium kept dark by a double cover of black cardboard, and 8 equally long defoliated stems were put at the same time into an aquarium exposed in the usual way to daylight. The base of the stems dipped into water. All conditions were equal except the illumination. After 23 days all the stems exposed to light had formed large basal roots and large shoots at the apex (Fig. 8). At the same time none of the stems

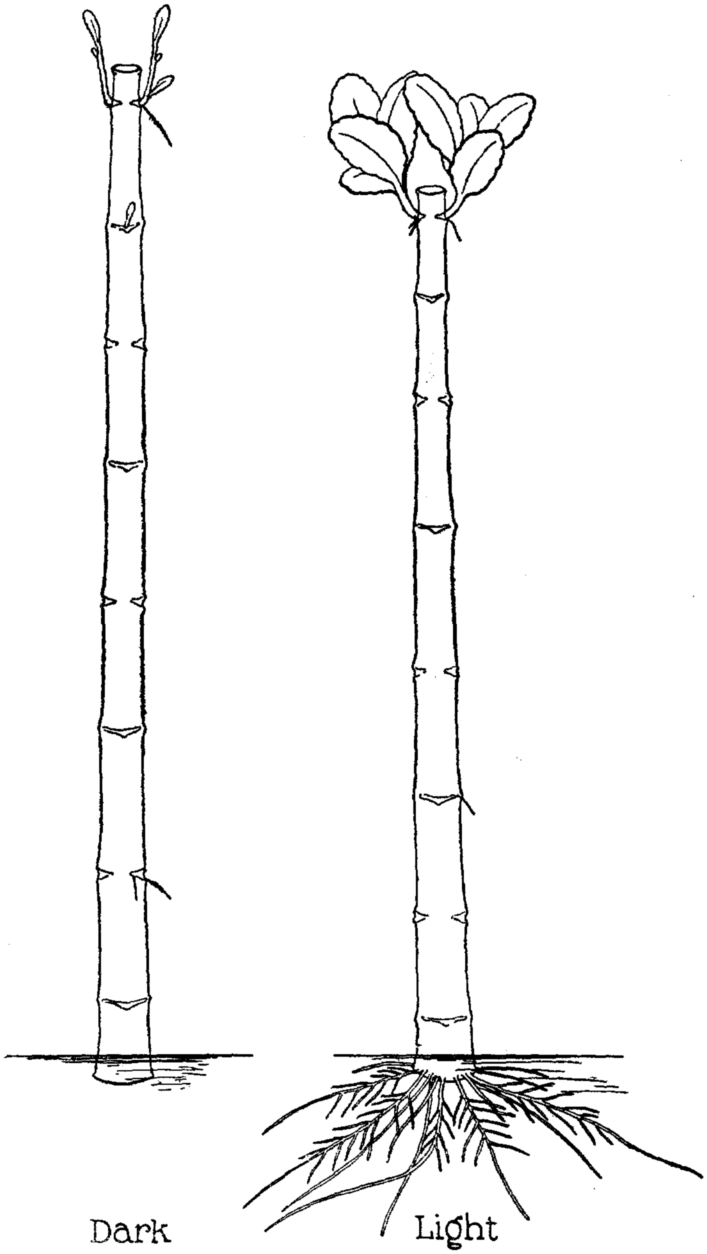

Fig. 8. Influence of light on root and shoot formation of stem. In the dark no roots are formed; in light ample roots are formed. The mass of shoots formed in dark is small compared with mass of shoots formed in light.

in the dark had formed a single basal root though some had formed tiny air roots (Fig. 8). The shoots formed in the dark had a small mass and the typical etiolated shape. The most striking phenomenon was the lack of root formation at the base of the stem in the dark. The writer had already shown that the favorable influence of the leaf on root formation in the stem also disappears when the leaf is deprived of light.[4]

SUMMARY AND CONCLUSION.

It is well known that a long defoliated piece of stem of *Bryophyllum calycinum* forms shoots only at the apical or the two apical nodes, while when such a stem is cut into as many pieces as there are nodes each node produces shoots. It is shown in this paper that the dry weight of shoots produced in the apical nodes of a long piece of stem is approximately equal to the dry weight of shoots the same stem would have produced if it had been cut into as many pieces as it possesses nodes. Hence all the material which can be used for the growth of shoots goes into the most apical part of the stem and this accounts for the polar character of regeneration in this case.[5]

It seems that the mass of basal roots produced by a piece of defoliated stem also increases with the mass of the stem.

[5] A plant morphologist, to whom the writer showed these experiments, commented that he was convinced that the shoot formation of an isolated piece of stem was due to a "stimulus." If we accept this suggestion, it follows that the "stimulus" for regeneration must have varied quantitatively with the mass of the defoliated stem in our experiments, and this would lead us again to the idea that the "stimulus" must be something material since it cannot well be spiritual.