

THE LAW CONTROLLING THE QUANTITY OF REGENERATION IN THE STEM OF BRYOPHYLLUM CALYGINUM.

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I.

It is well known that isolated pieces of plants and lower animals are able to replace the lost parts by a new growth which is called regeneration. The investigation of this phenomenon by merely qualitative methods of experimentation has led only to verbalisms. Thus it has been stated by Noll that the plant or animal possesses a "morphesthesia;" *i.e.*, a consciousness of what its proper form should be and hence the regeneration of lost organs. Driesch applies a similar verbalism calling the morphesthesia "entelechy." Weismann suggests that without the power of regeneration many species might have died out—hence regeneration. Others speak of regeneration as the effect of the "stimulus" of the wound. If we wish to substitute for these or similar expressions, which have led and can lead to no scientific result, a theory in the sense in which this word is used by the physicist, we must investigate the problem of regeneration by the methods of quantitative experimentation.

The writer has introduced such a method in the study of regeneration of the plant *Bryophyllum calycinum*, which is unusually favorable for work of this kind, and he has already reported some of the results obtained. The most significant fact was found in the regeneration of the leaf of this plant. When the leaf of *Bryophyllum* is detached from the plant, it will form roots and shoots in its notches. Each node of the stem has two leaves in opposite position—sister leaves—which under normal conditions have equal size. Since they have also the same age and the same history, they possess not only the same mass but contain also very probably chloroplasts in the same number and in the same degree of efficiency; so we can say such sister leaves

have approximately equal masses of *active* substance. The writer was able to show *that such sister leaves of equal weight produce equal masses of shoots in equal times and under equal conditions of illumination, temperature, and moisture*. He found, moreover, that if we reduce the mass of one set of sister leaves by cutting out pieces from the center of the leaves, while the other set remains intact, both sets produce shoots approximately in proportion to their masses, even if the number of shoots produced by the two sets differs widely. We, therefore, can say that *equal masses of sister leaves produce equal masses of shoots in equal times and under equal conditions, regardless of the number of shoots produced*.¹

This law shows that the problem of regeneration is part of the problem of growth and that it falls under the law of chemical mass action.

Inasmuch as age and previous history influence the active mass of photosynthetic material of the leaf, it is obvious that the law of the production of equal masses of shoots by equal masses of leaves is fulfilled more accurately by comparing sister leaves than it would be by comparing leaves with a different history; *i.e.*, leaves which differ in the mass of photosynthetic material in the unit mass of leaf. In such cases we should have to use statistical methods; *i.e.*, we should be compelled to use much larger numbers of leaves in order to eliminate the influence of variation in the relative mass of photosynthetic and other material in the leaf which is required for the regeneration (growth) of the shoots.

II.

Regeneration in an isolated piece of stem is much more general than regeneration in an isolated leaf, and the question arises whether a similar mass law, as that found for regeneration in a leaf, controls the quantity of regeneration in a stem.

In the axil of each leaf of the stem of *Bryophyllum* is found a dormant bud capable of growing into a shoot (3, Fig. 1). Each node contains two such dormant buds on opposite sides, one in the axil of each leaf. In successive nodes the lines connecting the two leaves

¹Loeb, J., *Science*, 1917, xlv, 436; *Bot. Gaz.*, 1918, lxv, 150; *Ann. Inst. Pasteur*, 1918, xxxii, 1.

and their axillary buds are at right angles to each other. If we, therefore, split a stem longitudinally into two halves (Fig. 1) in such a way that the plane of section is at right angles to the line connecting the two most apical buds (1, Fig. 1), the section cuts through the buds in the next node (2, Fig. 1), and injures them more or less, while it leaves the buds of the second next (3, Fig. 1) node intact, and so on. It happens for reasons which need not be discussed here that in such a stem as a rule only the most apical bud (1, Fig. 1) grows out.

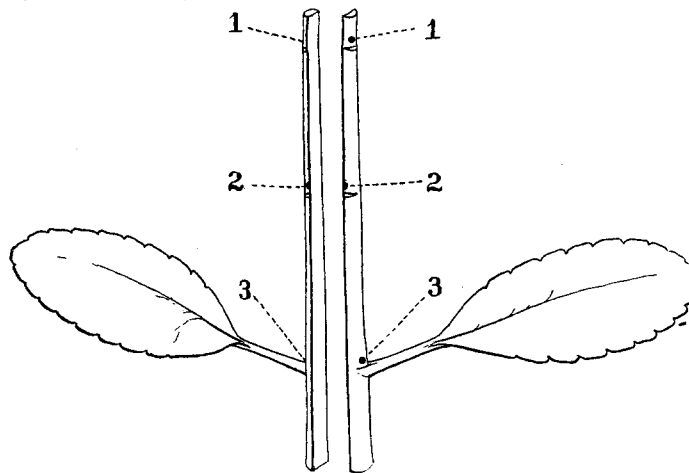


Fig. 1. Diagram showing the method of splitting the stem longitudinally in order to investigate the influence of mass of basal leaf upon shoot production. 1, 2, 3, designate the three pairs of dormant buds, two in each of the three nodes of the stem.

The following method was used for testing whether or not the quantity of regeneration in a piece of stem is controlled by the simple mass law which holds for the regeneration of shoots in the stem. From the stem of *Bryophyllum* were cut pieces containing three nodes (Fig. 1). All the leaves were removed except the two at the basal node. Then the stem was split lengthwise so that each half of the stem contained one basal leaf and one intact bud at the apex (1, Fig. 1), while the two buds (2, Fig. 1), in the middle node were generally injured in the operation. As stated, in most cases only the apical bud grows out in such a piece of stem. By leaving the leaf in one of

the half stems intact, while the sister leaf at the base of the other half stem is reduced in size, it is possible to find out whether the mass of the shoots regenerated at the apex of each half stem bears any relation to the mass of the two leaves. Such experiments were made by us and as a rule six or more different stems were used in one experiment.²

Many experiments are lost for the reason that the leaf at the base of a stem wilts or perishes much more rapidly than a leaf entirely detached from a stem; the latter may last for months while the former will usually wilt after a few weeks. For this reason it is more difficult to obtain exact results in investigating the influence of the mass of the leaf on the mass of the shoots formed at the apex of a stem than in investigating this influence upon the mass of shoots regenerated by isolated leaves. But in watching the condition of the leaves and realizing that we can only utilize an experiment when all the leaves remain intact we are able to obtain reliable results. This source of error due to wilting and decay of the leaf attached to the base of a stem which restricts the duration of the experiments is greater when the thin summer leaves are used than when the more fleshy and more durable winter leaves are utilized.

We shall first give the numerical results of some experiments in which only the fresh weight of the leaves and of the shoots regenerated by the stem was ascertained (Table I).

In Experiment 3 of Table I, the leaves of both half stems were left intact; 1 gm. of leaf in one set caused the production of 213 mg. of shoots in the stem, while 1 gm. of leaf of the other set produced 240 mg. of shoots in the apex of the stem. This shows the degree of accuracy to be expected in these experiments.

In the three other experiments the masses of the two sets of sister leaves varied considerably. Thus in Experiment 1 the masses of the leaves were approximately in the ratio of 1 : 6 (2.8 : 19.0); the masses of shoots produced by the two sets of stems were also approximately in the ratio of 1 : 6, namely 0.44 : 2.8. Experiment 2 gave similarly good results. The ratio of the two masses of sister leaves was approximately 1 : 5, namely 3.5 : 18.5, and the masses of shoots produced by the stem were also approximately in the ratio of 1 : 5, namely 0.7 : 3.6.

² Loeb, *Proc. Nat. Acad. Sc.*, 1918, iv, 117.

In Experiment 4 the two masses of sister leaves were in the ratio of almost 1 : 5, namely 2.7 : 11.9, and the masses of shoots produced by the stem were also approximately in the ratio of 1 : 5, namely 0.53 : 2.7.

Our experiments have shown without exception that the greater the mass of a basal leaf the greater the mass of shoot regenerated by the apex of the stem in equal times and under equal conditions; and wherever we were certain that the leaves remained normal during the experiment it was also possible to show that the mass of shoots produced by the apex of the stem

TABLE I.

Influence of Mass of Leaf at Base of a Piece of Stem upon Mass of Shoot Regenerated at the Apex of the Piece of Stem. Apices of Leaves Dipping in Water, Stems Suspended in Moist Air (See Fig. 2).

No. of experiment.	Duration of experiment.		Fresh weight of leaves.	Fresh weight of 6 regenerated shoots on stem.	Regenerated shoots per gm. of leaf.
	<i>days</i>		<i>gm.</i>	<i>gm.</i>	<i>mg.</i>
1	37	6 whole leaves.....	19.030	2.808	146
		6 sister " reduced in size.....	2.853	0.443	152
2	34	6 whole leaves.....	18.490	3.586	194
		6 sister " reduced in size.....	3.503	0.668	186
3	26	6 whole leaves.....	7.128	1.511	213
		6 " sister leaves.....	8.142	1.963	240
4	33	6 whole leaves.....	11.878	2.728	229
		6 sister " reduced in size.....	2.740	0.530	200

varied approximately in direct proportion to the mass of the leaf attached to the base of the piece of stem.

Fig. 2 is a drawing of the appearance of a group of six pairs of half stems having a leaf at the base. The duration of the experiment was 21 days. The upper half stem with a whole leaf is always the sister piece of the half stem with a reduced leaf immediately below it. It is obvious that the stems with the reduced leaves have smaller shoots than those with whole leaves. The apices of the leaves were dipped in water, the stems were in moist air. Fig. 3 is a photographic

reproduction of the same experiment (with left and right sides reversed).

These experiments prove that *equal masses of leaves at the base of a piece of stem cause the production of approximately equal masses of shoots at the apex of the stem in equal times and under equal conditions.*

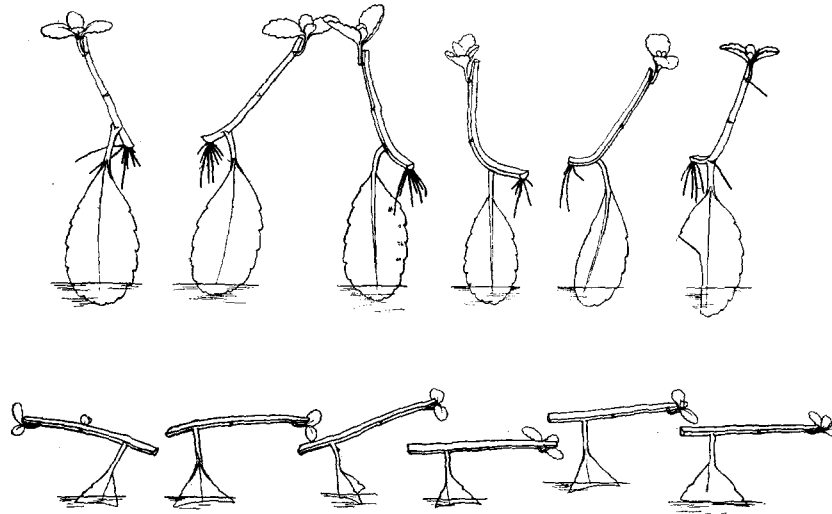


Fig. 2. Drawn after nature. The upper specimen with whole leaf and the one beneath it with reduced leaf are always the two halves of the same piece of stem split lengthwise in the way indicated in Fig. 1. The shoots at the apex of the stem with whole leaves (upper row) are considerably larger than the corresponding shoots with the sister leaf reduced in size (lower row). As a matter of fact, the masses of regenerated shoots were in proportion to the masses of the leaves attached to the base of the stems. The reader will also notice that the stems with whole leaves (upper row) have formed roots at their base, while those with reduced leaves have not yet formed any roots (though they did so later). It is also noticeable that the shoots with whole leaves show geotropic curvature, while those with reduced leaves are still straight.

Even where some of the leaves were wilted the mass of shoots produced at the apex was always greater when the mass of leaves was greater, but it was no longer possible to prove approximate proportionality though such proportionality in all probability existed.

In the experiments presented in Table II the stems as well as the leaves were suspended in moist air to eliminate the influence of ab-

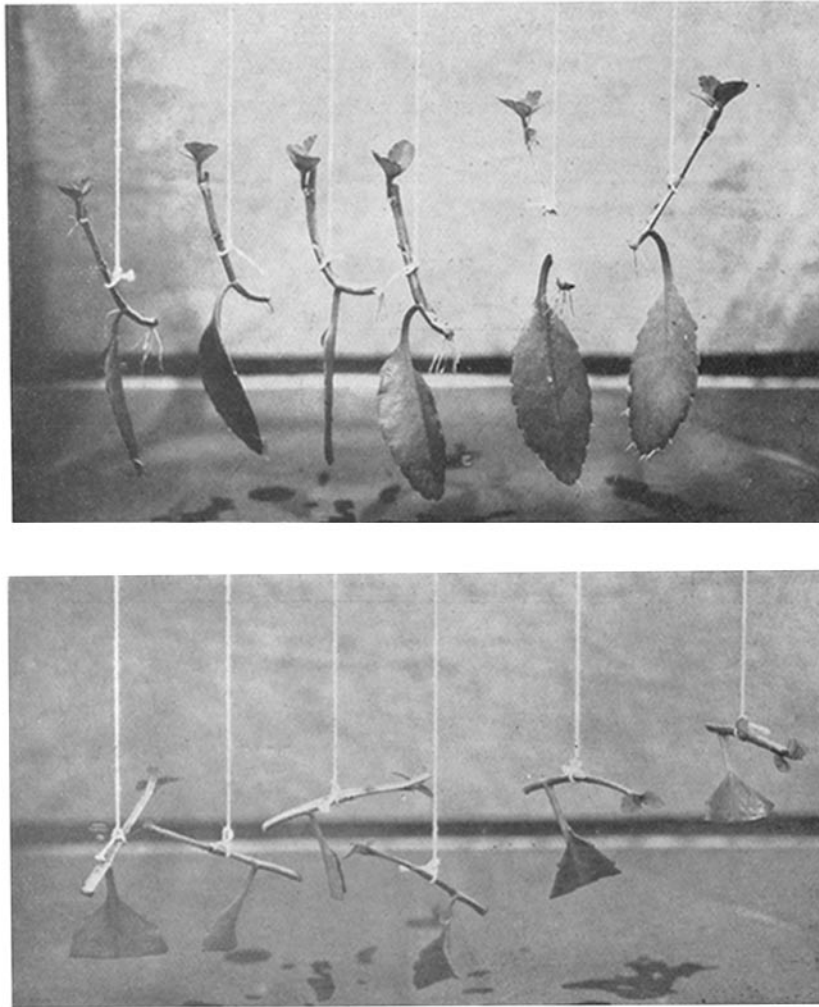


Fig. 3. Photograph showing the same experiment as in Fig. 2. (Left and right are reversed in Figs. 2 and 3.)

sorption of water by the leaf. The duration of these experiments had to be short on account of the more rapid wilting of the leaves connected with the stems.

In this case the disturbing factor of wilting is greater than when the apices of the leaves dip into water and hence the results with leaves suspended entirely in air show a smaller approximation to proportionality between mass of leaves and mass of shoots produced by the stem than that demonstrated in Table I, although the proportionality is not entirely obliterated.

TABLE II.
Stems and Leaves Suspended in Moist Air.

No. of experiment.	Duration of experiment.		Fresh weight of leaves.	Fresh weight of 6 regenerated shoots on stem.	Regenerated shoots per gm. of leaf.
	<i>days</i>		<i>gm.</i>	<i>gm.</i>	<i>mg.</i>
1	17	6 whole leaves	4.376	0.524	119
		6 " sister leaves.....	3.264	0.494	151
2	19	6 whole leaves.....	6.662	1.126	168
		6 sister " reduced in size.....	2.853	0.6885	237

III.

The mass of shoot produced in an isolated piece of stem entirely deprived of leaf is small compared with that produced when a leaf is attached to the base of the stem. This was demonstrated in the following experiment.

Six stems, each containing three nodes and one leaf at the base, were split longitudinally in the way described above. One half stem contained one leaf at the base, the other had no leaf. To insure an equal water supply to the stems they were put with their cut sides on moist filter paper (Fig. 4), the leaf at the base of the one set of stems being in moist air. The duration of the experiment was 24 days.

Fresh weight of shoots produced in 6 half stems *without leaves*, 0.120 gm. (dry weight, 0.0105 gm.).

Fresh weight of shoots produced in 6 half stems *with leaves*, 2.088 gm. (dry weight, 0.2015 gm.).

Weight of 6 leaves, 8.262 gm.

The dry weight of the 6 stems without leaves was 0.286 gm. and the fresh weight was 3.935 gm. The dry weight of the 6 half stems containing a leaf was 0.688 gm. and the fresh weight 6.203 gm. We have already shown in a previous paper that the mass of an isolated piece of stem increases when a leaf is attached to it.³

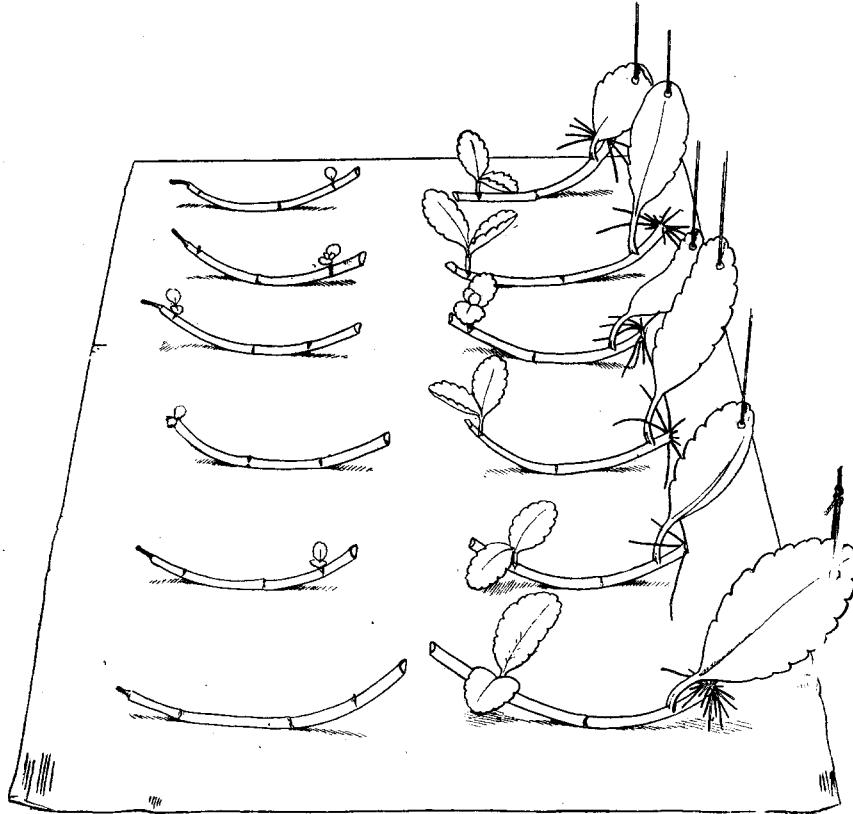


Fig. 4. Showing the enormous difference in mass of shoots regenerated by a half stem with leaf at base and the other half stem without leaf. The stems rested with their cut surface on moist filter paper.

Hence we can say that only about 5 per cent of the material of the regenerated shoots was contributed by the stem and about 95 per cent of the mass of the regenerated shoots of the stem was formed from

³ Loeb, *Ann. Inst. Pasteur*, 1918, xxxii, 1.

material furnished by the leaf. This is not surprising since we know that the leaf is the organ where the material for new growth in the plant is manufactured. It is also to be expected that in pieces of stems of different mass but without leaves the mass of shoots regenerated will increase with the mass of the piece of stem. The writer has already published experiments indicating that this is true,⁴ though he has not yet made quantitative determinations to find out whether the law of proportionality holds in this case also.

IV.

The law of proportionality between mass of the leaf attached to the base of a stem and mass of shoot produced apically from the leaf can be proved for other cases also. If we cut out a piece of stem with only one node containing two leaves such a piece possesses only two buds capable of developing into shoots; namely, one in each of the axils of the two leaves (Fig. 5). These axillary buds grow out more rarely and more slowly than the free buds at the apex of a piece of stem. Fourteen pieces of stem with one node and two leaves each were cut out from plants and each piece of stem was split longitudinally between the two leaves. One leaf remained always intact, the other leaf was reduced by cutting off part of the leaf (Fig. 5). Eight of these fourteen pieces of specimens formed axillary shoots. It seemed of interest to find out whether the mass of these shoots was approximately in proportion to the mass of the leaves. This was the case (Fig. 5). The duration of the experiment was 45 days. The apices of the leaves dipped into water.

a. Weight of 8 whole leaves, 10.968 gm. Weight of 8 shoots produced in their axil, 1.8025 gm. Mg. of shoots produced per gm. of leaf, 164.

b. Weight of 8 reduced sister leaves, 3.586 gm. Weight of 8 shoots produced in axil, 0.5895 gm. Mg. of shoots produced per gm. of leaf, 164.

The mass of axillary shoots produced by each set of sister leaves was, therefore, in direct proportion to the mass of the leaves.

⁴ Loeb, *Bot. Gaz.*, 1915, ix, 249.

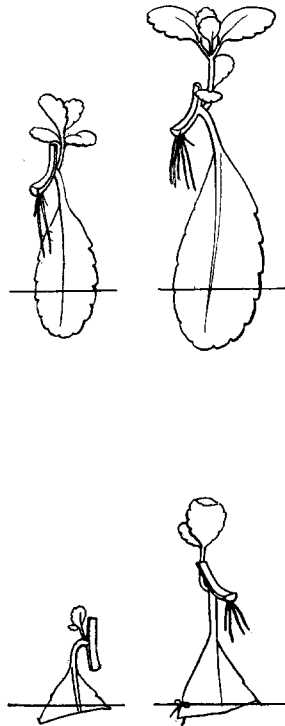


Fig. 5. Showing influence of mass of leaf on mass of shoot produced in the axil of each leaf. Each upper specimen with whole leaf and the specimen below it with reduced leaf are halves of the same stem.

V.

Since it might be argued that the leaves furnish only water for the growth of the shoots, it was necessary to find out whether the dry weight of the shoots regenerated by the stem varies also with the dry weight of the leaves attached to the base of the stem. The dry weight of the basal leaves and of the shoots regenerated by the stem was ascertained by drying these organs in an electric oven over night at 100°C. Some of the experiments mentioned in Tables I and II were used for this purpose and in Table III the experiments in which the dry weights were determined are indicated.

The leaves, therefore, do not only supply water for the regeneration of the shoots by the apex but dry matter as well. This conclusion is supported by the experiments to be discussed in the next chapter.

TABLE III.

No. of experiment.		Dry weight of leaves.	Dry weight of shoots.	Dry weight of shoots per gm. of dry weight of leaf.
		<i>gm.</i>	<i>gm.</i>	<i>mg.</i>
4, Table I.	6 whole leaves.....	0.440	0.2135	0.485
	6 sister " reduced in size.....	0.1055	0.0430	0.407
2, Table II.	6 whole leaves.....	0.3700	0.1002	0.271
	6 sister " reduced in size.....	0.1991	0.0632	0.317
1, Table II.	6 whole leaves.....	0.3144	0.0486	0.154
	6 " sister leaves.....	0.2585	0.0450	0.177

VI.

If it is true that the leaf furnishes the material to the stem from which the regenerating shoot grows, it should be possible to show that the basal leaf in connection with a regenerating stem has (after some time) less weight than the sister leaf which is separated entirely from the stem. This can indeed be demonstrated. A piece of stem is cut from a plant and all the leaves are removed except the two leaves at the basal node of the stem. One basal leaf is entirely separated from the stem to serve as a control; the other leaf remains in connection with the stem.

Six stems, about 6 cm. long, with several nodes and with a basal pair of leaves of approximately or practically the same size were selected for the experiment. Such sister leaves of the same size have also practically the same mass as shown in previous experiments. Both the isolated leaves and the stems with one leaf attached were suspended in moist air, in the same aquarium under identical conditions of light, temperature, and moisture. After 16 days the fresh and dry weights of the two sets of leaves, namely of the detached leaves and of their sister leaves connected with the stem, were ascertained. It was found that the leaves connected with the stems weighed considerably less than the detached leaves, and the difference was far in excess of the natural variation in the weight of fresh sister leaves of equal size.

Experiment I. Leaves and Stems in Moist Air.

	Fresh weight. gm.	Dry weight. gm.
6 detached leaves, at end of experiment	8.242	0.826
6 sister leaves connected with stem, at end of experiment	6.476	0.578

Hence the leaves connected with the base of the stem had given off to the latter about one-third of their dry weight.

During this time the detached leaves formed roots and tiny shoots in some of their notches while nothing of this kind occurred in the leaves connected with the stems. Instead twelve shoots were formed at the apices of the six stems and the twelve shoots weighed fresh 0.528 gm. and dry, 0.068 gm. The inference is that the material for the latter came from the leaves, but apparently more material than this was given off by the leaves to the stems. We have indeed shown in a previous paper that aside from the material for shoot formation the leaf sends material into the stem which may be used for the growth of certain tissues in the stem, resulting in callus formation, geotropic curvature, and increased thickness of the stem.³

The experiment was repeated with this difference, that the apices of the leaves dipped into water, while the stem and the rest of the leaves were suspended in moist air. Both sets of leaves were in the same aquarium under equal conditions of temperature, light, and moisture, and both were of equal size and mass at the beginning of the experiment. The experiment lasted 18 days.

Experiment II. Apices of Leaves Dipping in Water; Stems Suspended in Moist Air.

	Fresh weight. gm.	Dry weight. gm.
6 detached leaves, at end of experiment	11.159	0.791
6 sister leaves attached to base of stem, at end of experiment	4.485	0.388

Hence the leaves gave off to the stem about 50 per cent of their dry weight.

This difference is only partially accounted for by shoot production in the stem, the total shoot production amounting to 1.166 gm. fresh and 0.111 gm. dry weight.

Many more experiments than these were made and in some the leaves were weighed both at the beginning and at the end of the

experiment.⁵ They leave no doubt of the fact that the basal leaf gives off a considerable amount of its material to the stem.

We have, therefore, a right to conclude that a basal leaf gives off material to the stem, part of which is used for the formation of shoots in the stem.

Experiments were carried on in the dark and it was found that the mass of shoots regenerated under these conditions was small even when a large basal leaf was attached to the stem; and no proportionality between mass of leaf and of shoots regenerated on the stem could be found. This indicates that the products of assimilation in the leaf are part of the material used for the regeneration of the shoots on the stem.

Theoretical Remarks.

These experiments have shown that in the case of the regeneration of the leaf as well as of the stem the quantity of regeneration is determined by the mass of material sent out by the leaf and manufactured in the leaf; and possibly also to a small extent by material manufactured in the stem or circulating or stored in the stem at the time the stem was cut out from the plant.

The same simple mass law seems to hold for the quantity of roots regenerated at the basal end of the stem or basally from a leaf attached to the stem. It was very obvious in all our experiments that the mass of roots formed in a piece of stem in a given time increases with the mass of a leaf and the roots commence to grow out later when the leaf is smaller. The writer has, however, not yet made enough quantitative measurements to permit him to state that there is a strict proportionality between mass of leaf and mass of roots regenerated.

In animals the blood and lymph play the same rôle as does the sap in plants, and we may surmise that the quantity of sugar, amino-acids, salts, and of "accessory substances" in the body fluids determines the quantity of regeneration in animals. In an animal, regeneration may occur even when no food is taken up, and it is to be assumed that the tissues of a fasting organism constantly convert some of the material stored in the cells into sugar, amino-acids, and vitamins,

⁵ We will omit these experiments here since they will be discussed in one of the following papers.

which diffuse into the blood and become available for the growth of the regenerating tissues; in other words, it is the constant hydrolysis going on in the organism which supplies the material for the growth and regeneration of fasting animals. It remains for future investigations to find out whether the ratio between mass of growth material circulating in the blood or lymph and the quantity of regeneration obeys also the simple mass law established for regeneration in *Bryophyllum*.

For those interested in the dynamics of these processes attention may be called to the fact that in our experiments the action of two masses of active material, m and m_1 , in two sister leaves is compared. These masses are certain constituents of the two sister leaves, primarily the chlorophyll, and their ratio $\frac{m}{m_1}$ may be considered approximately constant throughout the duration of the experiment. Our experiments have furnished the proof that the ratio of the mass of shoot regeneration in two halves of a stem (each possessing a leaf at the base) is approximately proportional to $\frac{m}{m_1}$; or in other words, that the law controlling the quantity of shoot regeneration of the stem is a special case of the law of mass action.

SUMMARY.

1. A method is given which allows us to measure the influence of the mass of a leaf upon the quantity of shoots regenerated in an isolated piece of stem. This method consists in isolating a piece of stem with only two leaves left at the basal node and then splitting the stem lengthwise so that each half has one basal leaf. By leaving one leaf intact while the size of the sister leaf is reduced, the influence of the mass of the leaf upon the quantity of shoots regenerated by the stem can be measured.

2. This method has yielded the result that the mass of shoots regenerated at the apex of such a piece of stem increases under equal conditions and in equal time with the mass of the leaf, and is approximately proportional to the mass of the leaf.

3. Such an influence of the mass of the leaf upon the mass of shoots produced by the stem is only intelligible on the assumption that the

growth of the regenerating shoot occurs at the expense of material furnished by the basal leaf.

4. This assumption is supported by two facts: first, that in the dark this influence of the leaf disappears more or less completely; and, second, that a leaf attached to the base of a regenerating stem after some time weighs markedly less than does a sister leaf completely detached from the stem, but otherwise under equal conditions.

5. This latter fact that a leaf when attached to the base of an excised piece of stem wilts more rapidly than when completely isolated is the reason that the proportionality between mass of a basal leaf and mass of shoot regenerated at the apex of an isolated piece of stem cannot always be demonstrated with the same degree of accuracy as the proportionality between the mass of completely isolated leaves and the mass of shoots they produce.

6. The material furnished by the leaf to the stem is not restricted to water but includes also the solutes, since not only the fresh weight but also the dry weight of the shoot regenerated by a piece of stem increases with the mass of the leaf attached to the base of the stem; and since not only the water contents but the dry weight of a leaf attached to the base of an excised piece of stem diminish when compared with the dry weight of a completely detached sister leaf.

7. The mass of shoots produced by an isolated piece of stem without leaf is small and almost negligible compared with the mass of shoots produced by the same piece of stem when a leaf of sufficient mass is attached to the base of the stem.