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THE CHEMICAL BASIS OF AXIAL POLARITY IN REGENERATION

I

WHEN a piece of a stem is cut out from a plant one or more new shoots will usually arise at the apical, and roots at the basal end of the piece. This phenomenon of axial polarity was explained by the older botanists as being due to a flow of shoot-forming substances to the apex and of root-forming substances to the base. The gathering of these substances at opposite ends of the piece was believed to be responsible for the phenomenon of polarity in regeneration. While this may or may not be correct, the writer has recently found facts which suggest an additional or a different mechanism for this polarity, namely, that the apical bud suppresses the growth of the buds situated more basally in the stem by sending out inhibitory substances in a basal direction.

The experiments were made on *Bryophyllum calycinum.* Each node of the stem of this plant has two leaves in an opposite position, and in the axil of each leaf is found a dormant bud capable of giving rise to a shoot. The line connecting two buds of one node is at right angles to the line connecting the two buds of the next node.

Experiment I.—A piece of stem, containing six or more nodes, is cut out from a plant, all the leaves are removed and the piece is put into a horizontal position with the line connecting the two buds of the most apical node vertical. In this case both buds in the apical node may begin to grow, but as a rule only the upper bud will continue to grow, while the growth of the lower bud will soon stop altogether or will be considerably retarded. None of the buds in the other nodes will grow out. Roots will grow chiefly on the under side of the stem, but in the last node and at the cut end they may form on the upper side as well as on the lower side of the stem.

Experiment II. is the same as Experiment I., except that the upper apical bud is cut out. In this case the lower apical bud will grow rapidly, but in addition one or both of the buds of the node next to the apical will grow out. These buds never grow out when the upper apical bud is preserved and healthy.

Experiment III. is the same as the previous experiment except that the lower apical bud is removed, while the upper one is preserved. In this case, the upper apical bud will grow out, but none of the others.

It follows from these experiments that the upper apical bud inhibits or retards the growth of the lower apical bud as well as that of the rest of the buds; while the lower apical bud can not suppress the growth of the buds in the node behind. The writer has repeated these experiments in many modifications, among which those on longitudinally split stems are the most striking. The results were uniform.

All these observations are intelligible if we assume that a bud when it begins to grow produces and sends out inhibitory substances toward the base of the stem. These substances flow in the conducting vessels in the same half of the stem where the bud lies; when one apical bud is above and one below, the two buds in the next node are in a lateral position between the upper and lower half of the stem. Hence the inhibitory substances sent out by the upper apical bud can reach the two buds in the next node behind and inhibit their growth, since these buds lie directly below or on the lower level of the conducting vessels from the upper apical bud; while inhibitory substances sent out by the lower apical bud can not reach the buds in the node behind in large quantity, since these buds are on the upper level or slightly above these conducting vessels. When the two lateral buds grow out they will inhibit the growth of all the buds behind, each bud covering a territory of one half stem.

The alternative hypothesis assumes that since the apical bud is the first to grow out it will absorb all the shoot-forming material.[1] If we assume that the shoot-forming material has a tendency to rise this hypothesis may explain the facts also. But the following experiment, which seems crucial, decides in favor of the other assumption.

A piece of stem containing a number of nodes is suspended horizontally, as in the previous experiments, with the two apical buds in a vertical line. All the leaves are removed with the exception of those at the apical node. Here the petioles of the leaves are left attached to the stem, the leaves having been cut off. The petioles will wilt in a week or ten days, but until then will prevent or retard the growth of the apical buds in their axils. The buds in the next node will begin to grow out and as soon as the petioles have fallen off the apical buds will also begin to grow.

The next step is decisive for testing the two hypotheses. If the inhibiting effect of the apical buds on the more basal buds is due to the fact that the buds which grow out first attract all the material from the basal part of the stem, the buds in the node behind the apical one, which grew out first, should continue to outstrip in growth the apical buds which began to grow out later. But if the inhibiting effect is due to an in-

[1] This form of inhibition exists apparently in the leaf where the shoots which grow out first prevent other notches in the leaf from giving rise to shoots by absorbing the material needed for shoot formation. SCIENCE, 1917, XLV., 436; XLVI., 115; *Bot. Gaz.*, in print.

hibitory substance being sent in the direction toward the base by the growing bud, the most apical bud should soon outstrip in growth those situated in the next node behind, although the latter had an earlier start. For according to this theory, the most apical buds should be sending substances toward the base which inhibit the growth in the next bud; while the most apical buds receive no such inhibitory substances. The results of the experiment are quite clear. As soon as the petioles at the apex fall off the axillary buds at the apex begin to grow out and soon not only outstrip in size those of the next buds behind but actually retard or stop the growth of the latter. This phenomenon seems intelligible only on the assumption that a growing bud sends out substances toward the base of the stem which directly inhibit the growth of the other buds.

II

If the inhibition of shoot formation is due to special inhibitory substances it should be possible to show that the inhibition varies quantitatively with the mass of inhibitory substances produced in the growing bud, or with the mass of the latter. While the bud is too small for convenient quantitative experimentation, it can be carried out satisfactorily with the leaf. In a former paper the writer had shown that the leaf of *Bryophyllum* sends out material toward the base of the stem which favors root formation; and it also seemed possible that the leaf might send out substances in a basal direction which inhibit shoot formation. The sap from the leaf flows in conducting vessels situated in the same half of the stem where the leaf is attached.

When we suspend a stem of *Bryophyllum* with six or more nodes horizontally, and remove all the leaves except the two in the apical node, the stem will form no shoots as long as the leaves are alive, but an abundance of roots is produced in the stem. The two leaves, therefore, inhibit all the shoot formation in the buds situated basally from the leaf. When we remove one of the two apical leaves the axillary bud of this leaf will grow out and it will have the same inhibiting effect as the leaf in the previous experiment. We now make the following experiment.

Twelve long stems from which all leaves except one of the two apical ones have been removed are suspended horizontally, and the free axillary bud opposite the leaf is also cut out. Six stems are suspended with the leaf above, six with the leaf below. There is a striking difference in the two sets. When the leaf is below, shoots will develop either in the two lateral buds of the first node behind the leaf, or on the upper side of the second node behind the leaf. When the leaf is above, no shoots will develop in the next node behind the leaf but one shoot may grow in the second node behind the leaf, *on the lower side alone.* These shoots will develop more slowly than those in the stems whose leaf is on the lower side.

This is exactly the result which we should expect if the leaf sends out substances inhibiting shoot formation toward the base of the stem. These substances, being identical with or accompanying the root-forming substances, flow on that side of the stem where the leaf is, but have naturally a tendency to flow downward and not to flow upward. Hence, when the leaf is below it is possible for shoots to form in some (about 50 per cent.) of the stems in the first node behind the leaf, in which case the buds are on the upper level of the flowing sap; while when the leaf is above it is impossible for the buds in the first node behind the leaf to grow because they are on the lower level of the sap flow from the leaf. The bud on the lower side of the second node behind the leaf (when the latter is on the upper side of the stem) is outside the sap flow and hence it may develop.

When we work with a large apical leaf attached to a short stem (the free apical bud opposite the leaf is always removed in these experiments) containing only two nodes behind the leaf, everything is as described for long stems. When, however, the piece of stem behind the leaf is smaller, containing only one node, no shoot can grow on this stem even when the leaf is below. The mass of inhibitory substance sent out by a large leaf will flood the buds in this node with inhibiting material. Occasionally a bud starts to grow but stops before a leaflet has time to unfold. Such a stem will form an abundance of roots at the base. If, however, we reduce the size of the apical leaf by cutting away nine tenths of its mass, most or practically all the stems will form shoots in the node behind the leaf; but roots in such stems either do not develop at all or only with long delay.

The leaf, therefore, sends substances to the basal part of the stem which inhibit shoot formation and favor root formation, and the mass of these inhibitory substances decreases with the mass of the leaf, and apparently parallel with the mass of root-forming substances sent to the base of the stem.

Another experiment is equally instructive. We have seen that when long stems having all but one apical leaf removed (and the opposite free apical bud also removed) are suspended horizontally, with the leaf above, no shoot will form on the upper side of the stem. When we reduce the size of the leaf sufficiently this inhibition ceases.

Again the objection might be raised that the inhibiting effect of the leaf on shoot formation in the region behind the leaf is due not to an inhibitory substance being sent out by the leaf but by nutritive substances needed for the growth of shoots being sent into the leaf by the stem. This is highly improbable not only on the basis of our knowledge of these processes but also on account of the following fact. When we cut off a leaf without its petiole, leaving the latter in connection with the stem, the petiole will dry out and fall off in a week or less. If, however, the petiole is detached from the stem but left attached to a leaf, it will not wilt, but remain fresh and green as long as the leaf is alive, which may be many months. This shows that nutritive material is furnished by the leaf to the stem, and not vice versa.

III

While these experiments show that the inhibiting influence of an apical bud on the growth of the more basal buds is due to one or more inhibitory substances being sent toward the basal end of the stem, the other main fact of polarity remains unexplained; namely, how it happens that the most apical bud grows out first. The writer is inclined to offer the following suggestion: In the normal plant, the substances inhibiting shoot formation are constantly flowing from the growing region toward the root of the plant. When we cut out a piece of stem and remove the leaves these substances will at first exist in every node, but will continue to flow toward the base. Hence the most apical node will be the first one to be free from these inhibitory substances and the bud or buds situated here can now begin to grow out. As soon as they grow out they will maintain a constant flow of inhibitory substances toward the base which will suppress the growth of buds in the more basal part of the stem.

The experiments, therefore, seem to prove that axial polarity in the regeneration of a stem is due to the fact that the apical bud (as well as an apical leaf) send out substances toward the base of a stem which inhibit the buds from growing out. These inhibitory substances may be identical with or may accompany the root-forming hormones. The most apical bud in an excised piece of stem will grow out first since it will be the first to be free from these inhibitory substances.

In a former paper the writer had pointed out that a leaf sends out substances, in an apical direction through the stem, which favor shoot formation.

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SOME COMMENTS ON THE THEORIES OF THE STRUCTURE OF MATTER[1]

PROFESSOR LEWIS in his paper raised the question of valence. From the point of view of chemistry, valence has a definite meaning which can not be overlooked and which may be emphasized here. The conception of valence developed from a study of the regularities observed in the composition of substances, and is fundamentally purely descriptive. It is a classification which shows regularities in the capacity of certain atoms for combination, or for holding a definite number of atoms or their equivalents in combination. The continued study of chemical composition has, as a matter of course, extended the classification. The phenomena of oxidation, the ionization of substances in solution and otherwise, and similar properties, have brought forward the view that, choosing a suitable element or state of an element as the zero or neutral point, the valence of an element in a given combination may be denoted either by a positive number or a negative number. This view was adopted for individual cases some time ago by different chemists, but became of general interest when J. J. Thomson, using corpuscles, showed how this could be pictured readily, and applied in a simple manner.

A few words may be devoted to the fact that the classifications given by valence should involve no considerations of measures of relative stabilities of substances, although the existence of compounds depends upon stabilities and rates of decomposition. Stability discussions should not enter directly into questions of valence, but unfortunately this fact is often overlooked and much confusion has resulted.

The question of so-called polar and non-polar valence is one raised by Professor Lewis. At the present time the view that only non-polar bonds exist is probably held by no chemist. The electron conception of valence, based upon a study and comparison of organic and inorganic compounds, postulates polar valence only; in other words, each valence linking is equivalent to one atom functioning with a negative charge, and the other atom with a positive charge. The electrostatic view does not involve at first sight such questions as distribution of electrons within the atom, etc.

At the present time there are a number of chemists who advocate both polar and non-polar valences, even assuming both to be present in a molecule at the same time. The reasons for assuming the existence of non-polar valences appear to be negative ones. If direct evidence is lacking, or if ignorance is manifested with regard to the reactions of certain groups, or if these groups do not take part in the desired reaction with sufficient velocity, the existence of polar valences is denied. A strong argument in favor of assuming polar valences in organic compounds is, that if they are

[1] This discussion was presented by Dr. Falk at the "Symposium on the Structure of Matter," held at the meeting of the American Association for the Advancement of Science in New York City, December, 1916.