CHEMOTROPISM OF ROOTS.[1]

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THE chemotropism of pollen tubes and of fungus hyphae and the chemotaxis of various unicellular organisms have received considerable attention in the great mass of work which has been done on the sensitive activities of plants. There is probably a well-defined notion that terrestrial roots, which are known to be hydrotropic, are also chemotropic. The unequal distribution of minerals in the soil, and of decaying organic matter, can be thought of as furnishing opportunity for the development of chemical response as a biological adaptation.

In literature there seems to be no record of work done to test the ability of roots to respond to a chemical stimulus by changing their direction of growth. This test has now been made, and the results are recorded in the following pages.

I. THE ONE-SIDED APPLICATION OF CHEMICALS IN TUBES.

The first method employed consisted in applying to the tips of roots immersed in a liquid a chemical diffusing from the open mouth of a horizontal tube.

A considerable quantity of the ordinary Sachs's culture fluid was made, containing all the ingredients except the potassium nitrate. A solution of potassium nitrate was then made of such a density that when a drop of it was placed in the solution of the other ingredients of the culture fluid, the potassium nitrate would neither rise nor fall. Rather large capillary tubes were now made, 1.5^{cm} long by 1^{mm} internal diameter and closed at one end. These tubes were then filled with the solution of potassium nitrate by the use of the air pump. Next, a row of seedlings, fastened by strips of blotting paper and rubber bands to a bar of glass, were suspended across a glass cylinder, the roots being immersed in the culture fluid containing no potassium nitrate. The tubes filled with the potassium nitrate solution were now introduced into the jar of liquid, and so adjusted that their open

[1] Contribution 68 from the Botanical Laboratory of the University of Michigan.

ends were within a few millimeters of the root-tips, there being a tube for each root. Every few hours the position of the tubes of potassium nitrate was changed in order to follow the descent of the root-tips. This method will be recognized as an adaptation of that method first employed for other purposes by Pfeffer.[2]

Altogether eighty-seven seedlings of *Helianthus annuus* L. and seventeen seedlings of *Raphanus sativus* L. were employed, for periods ranging from twenty-four to forty-eight hours at 20° to 24° C., but without showing a response. The positive curves and the negative curves were no more numerous than one might observe in these roots growing in water.

Thinking that the amount of potassium nitrate might have been too small to act as a stimulus, glass vials holding 20^{cc} were used instead. The mouths of the vials were closed by plugs of cotton previously wet with the potassium nitrate solution. Here also the roots showed no curves that could be ascribed to the chemical.

The absence of response in the foregoing experiments might be ascribed to any of several conditions. It might be that the roots used were not chemotropic, though others might be. It might be that the sunflower and the radish were chemotropic to some chemicals, but not to the potassium nitrate; though the thought suggested itself at the outset that, if roots are chemotropic they are likely to respond to chemicals which form their necessary food. It might be that the potassium nitrate was not present in large enough proportion. It might be that the difference in chemical composition was not great enough on opposite sides of the root. It might be, finally, that, while the seedling had a full supply of all kinds of stored food, it would show itself indifferent, but would respond, when it needed mineral food from without. Manifestly the thing to do was to satisfy these conditions as fully as possible.

II. THE ONE-SIDED APPLICATION OF A CHEMICAL BY DIFFUSION THROUGH A MEMBRANE.

With a view to testing the roots of plants when the food stored in the seed had been exhausted, water cultures were

[2] Ueber chemotactische Bewegungen, etc. Untersuch. Bot. Inst. Tübingen 1: 367.

started in the plant house in a full culture solution, except for the absence of potassium nitrate. After the plants had produced secondary roots, fresh culture fluid of the same composition was exchanged for the old, and there was inserted in each jar a glass dish of solution of potassium nitrate, whose density was approximately the same as that of the other liquid, as determined by the means already described. These glass dishes of potassium nitrate, holding 30^{cc}, were closed by parchment paper, or by hardened filter paper, and the dishes were set with the membrane parallel with the main roots and 2 to 3^{mm} distant from them. The preparations were continued in the plant house for six weeks, or long after all stored food was exhausted, both fluids being renewed every ten days. The roots were shielded from the light to avoid heliotropic curving. The species used were *Raphanus sativus* L., *Fagopyrum esculentum* Moench., *Lupinus albus* L., and *Pisum sativum* L. There was, however, no chemotropism shown.

To make sure that the failure of potassium nitrate solution to act as a stimulus was not due to the relatively small quantity employed, the foregoing experiment was repeated in all its details, except that the two solutions employed were nearly equal in volume. The preparation was continued for three weeks, and the solutions were renewed in the middle of the period. The time of year was summer, and the growth was good.

It would seem as though this preparation would bring curves, if the seedlings employed were chemotropic. Here was a row of roots parallel with and within 2 or 3^{mm} of a parchment membrane 9^{cm} in diameter having different solutions on its opposite sides. It was as though the jar had been divided into halves by a parchment septum, and the roots suspended on one side close to the septum. One can hardly think that in nature conditions can be more favorable for the manifestation of chemotropism. And this preparation did show a certain response. Nine seedlings of *Lupinus albus* and forty-four of *Raphanus sativus* were used. The former showed no curves nor other response ; but the Raphanus showed a considerable number of primary and secondary roots bending toward the potassium nitrate, and a much greater growth of lateral roots on the same side. This greater growth on the side toward the parchment membrane was not mainly due to the effect of the curving of the roots into that position; but the lateral roots on that side had made a better growth than on the opposite side. This behavior recalls the similar results obtained with varying soils by Nobbe,[3] Stohmann,[4] Höveler,[5] and Frank,[6] who obtained a greater growth of lateral roots in richer soils.

Here at last was a result indicating chemotropism of roots. Yet the method left much to be desired. With some means to insure a greater inequality in chemical composition on the opposite sides of a root, much better responses might be obtained.

III. DIFFERENT CHEMICALS BROUGHT TO OPPOSITE SIDES OF THE ROOTS BY STRIPS OF FILTER PAPER.

The device next tried was to conduct liquids of different composition to the opposite sides of the root by means of narrow strips of thin filter paper. The seedlings were suspended from a bar of glass fastened horizontally across a damp chamber. In the bottom of the chamber were placed two little dishes, one containing distilled water, the other Sachs's culture solution. From one liquid a strip of filter paper, 2^{mm} wide, extended upward and adhered to one side of the root, while the opposite side was covered by a strip from the other liquid. The strips of paper were not allowed to touch one another. In some tests, the small dishes were placed above the level of the root tips, and the paper strips hung downward, touching the flanks of the root-tip and then diverging and ending in dishes of water below. Every few hours the strips of paper were adjusted so as to keep them in contact with the roots near the tip. In this form of experiment nineteen seedlings of *Lupinus albus* were used, but no curves resulted. The objection to this method is found in the difficulty of keeping the paper strips in contact with the sloping sides of the root-tip.

[3] NOBBE: Landwirthsch. Versuchsstat. **4**: 222. 1862; and **10**: 100. 1868.

[4] STOHMANN: Jahresbericht über die Forschungen der Agriculturchemie 1868-69.

[5] HÖVELER: Jahrb. Wiss. Bot. **24**: 294. 1892.

[6] FRANK: Bot. Zeit. **51**: 153. 1893.

IV. THE APPLICATION OF CHEMICALS IN BLOCKS OF GELATIN.

The preceding methods all proved themselves unsatisfactory. Yet they are worth incorporating in this record, for they are methods which would probably suggest themselves to any one pursuing this subject, and it is worth while to show the unsatisfactory results they bring.

Another method was conceived which seemed to promise better control of the application of a chemical to only one side of a root. This was the employment of gelatin as a vehicle for the various chemicals. It was thought that gelatin blocks might be made with solutions of salts, and these blocks might be brought against the roots, a block on each side. There was, however, the question as to the behavior of roots growing in gelatin, and their ability to respond to stimuli. Wacker[7] determined the retardation in the growth of some roots in water, while Sachs[8] and Němec[9] mention the fact that roots inverted in air and water do not return completely to their usual geotropic position. Experiments in this laboratory have shown that there is a greater retardation of growth of roots in water than in air or earth, and a greater retardation in gelatin than in water. At the same time, it was found that geotropic response is not so great in gelatin as in water, and not so great in water as in earth or air. Still growth proceeds apparently normally in gelatin, except for retardation; and geotropic responses follow stimulation, though somewhat slowly. It was decided, therefore, to try the use of gelatin.

Large, rectangular, oblong glass jars were selected to serve as damp chambers. They were lined with filter paper, dipping into water in the bottom. Paper boxes of suitable size were then made, and these laid on glass plates, while their sides were held rigid with wooden frames. Into these boxes, as molds, the warm gelatin mass was then poured, and allowed to solidify. The gelatin mass had been made by dissolving the dry gelatin in a watery solution of the salt to be used on the roots. The

[7] WACKER: Jahrb. Wiss. Bot. **32**: 71. 1898.

[8] SACHS: Arbeiten Bot. Inst. Würzburg **1**: 409. 1873.

[9] NĚMEC: Jahrb. Wiss. Bot. **36**: 89. 1901.

gelatin blocks as they came from the molds were 15^{cm} long, 9^{cm} wide, and 2.5^{cm} thick. The seedlings to be used were fastened in the usual way to a bar of white pine which was suspended across the damp chamber at a suitable height. In the bottom of the damp chamber a glass platform had been raised above the water, and on this the gelatin blocks were laid, each block with a glass plate for a backing. A little practice soon enables one, with a hand on each glass plate, to turn the gelatin blocks up against the row of roots; and while with one hand the two blocks of gelatin are held in place, with the other hand they are secured there by bringing against the glass plate supporting them bars of wood held in place by springs of rubber tubing.

A row of thirteen seedlings of *Lupinus albus* was first used between the blocks of gelatin, one of which was made with distilled water, the other with 0.28 per cent. dry salt of di-sodic phosphate (Na_2HPO_4). The seedlings were 5^{cm} to 7^{cm} long, the temperature 23°, and the period twenty-four hours. The dry gelatin was 6 per cent. of the whole mass.

At the conclusion of the experiment, all thirteen roots were found grown into the sodium phosphate gelatin, the angles of curvature being 45° on the average. The roots looked healthy, and growth had been good. Decisive as this result was, it did not demonstrate the precise cause of the curving. The result may have been traumatropism, or hydrotropism (osmotropism), or chemotropism.

The curves could hardly be due to the presence of copper or other metal in the distilled water, for water from the same bottle was used in the gelatin on both sides of the seedlings. Yet to make doubly sure, the next gelatin blocks were made with water twice distilled in flasks of Jena glass. In this test eleven seedlings of *Lupinus albus* were employed as in the preceding experiment. Here as before all roots turned positively into the gelatin containing the sodium phosphate, nearly all angles being 45° or over.

It might be thought that the curves were due to the injurious action of the sodium phosphate on the growing zone of the root, retarding growth there, and thus causing the tip to swing around to make the positive curve. This result would be the same as the effect of mechanically wounding a root in the elongating zone, as determined by Spalding.[10] It is to be noted, however, that to produce a positive curve by injury the sodium phosphate must act traumatically on the elongating zone, whereas we should expect it to act first on the more sensitive root apex, and produce a negative traumatropic curve. To test the relative sensitiveness of the root apex, and of the elongating zone toward a known injurious substance, a block of gelatin was made up with a 0.01 per cent. aqueous solution of crystals of copper acetate, while the gelatin used on the opposite side of the roots was made with distilled water only. Sixteen seedlings of *Lupinus albus* were used. After six hours, the temperature being 21° to 22°, the gelatin blocks were separated, and the roots were seen to be bending away from the side holding the copper salt. The gelatin was replaced and allowed to remain eighteen hours longer, when the experiment was ended. Thirteen of the sixteen roots were strongly negative toward the copper acetate, while the other three roots remained straight. All roots were living and had grown somewhat.

It is thus demonstrated that when an injurious substance is presented at the same time to both the elongating zone and the apex of a root, the negative traumatropism of the root will overcome the tendency to form a positive mechanical curve. It is thus demonstrated that the curves toward sodium phosphate in the former experiments were not traumatic.

There is still the possibility that the curves toward the sodium phosphate were due to a disturbance of the turgor of the root. The salt on the one side may have caused a shortening of the cells on that side by withdrawing water; or the low osmotic pressure of the water and the gelatin on the opposite side may have caused an inflow of water into the cells, and hence a lengthening on that side of the root.

To test the possibilities named in the foregoing paragraph, a gelatin block was made up with 3.5 per cent. watery solution of

[10] SPALDING: Traumatropism of roots. Annals of Botany **8**: 423. 1894.

cane sugar, and brought against one side of a row of roots, while the gelatin block on the opposite side was made up with distilled water. Twelve seedlings of *Lupinus albus* were used in a temperature of 22°, and the duration of the experiment was twenty-four hours. All roots grew straight.

Since in the last experiment the roots had on each side a gelatin solution of equal strength, it follows that on the side of the sugar solution there was an excess of osmotic pressure corresponding to more than 183.4^{cm} of mercury. This, however, effected no bending.

Another set of sixteen seedlings of *Lupinus albus* was set up between gelatin blocks, the one side having a 3.5 per cent. solution of cane sugar, and the other a 0.28 per cent. solution on the dry salt Na_2HPO_4. When the preparation was taken down twenty-five hours afterward, every root had grown into the sodium phosphate, the angles running from 30° to 75°.

These two experiments certainly show that the curves toward the sodium phosphate are not to be explained as osmotropism nor ascribed to any mere physical disturbance of the water content of the cells. If the curves are due neither to injury nor to the osmotic action of the sodium salt (osmotropism or hydrotropism), there remains probably but one explanation: We have here a true case of chemotropism of roots.

Accepting the foregoing results as demonstrating the positive chemotropism of the roots of *Lupinus albus* toward sodium phosphate, it would be interesting to know whether with strong solution of the same salt the root would show itself negatively chemotropic.

A row of ten seedlings, having on one side a gelatin block made with distilled water, and on the other side a block made with 2 per cent. dry salt of di-sodic phosphate, had all their roots killed within a few hours. Another row of ten seedlings, similarly treated except that the gelatin on one side was made up with a 1.5 per cent. solution of the sodium salt, showed, after twenty-four hours, all the roots bent into the gelatin containing the chemical. All of the roots except one were dead. All had grown sufficiently, however, to make the curve before dying.

A third row of ten seedlings of the same species, set up between gelatin and distilled water on one side and gelatin and 1 per cent. di-sodic phosphate on the other, gave eight roots bent into the gelatin containing the chemical, while the other two roots were straight. None of the roots were dead. In the twenty-four hours of the last experiment, the roots had grown but about 10^{mm}, though the temperature was 20° to 23°. This shows a retardation of growth to about 50 per cent. of what it would have been in air or soil.

The last series of three experiments demonstrates that by strong solutions of sodium phosphate the roots of this plant unable to turn in negative chemotropism are helplessly lured on to certain death. The case is similar to that of several free-swimming organisms, mentioned by Rothert[11], which swim into solutions of lethal osmotic strength.

Though the roots of *Lupinus albus* are positively chemotropic toward di-sodic phosphate, they do not display a like behavior toward all salts that are absorbed by plants as food. Seedlings of *Lupinus albus* to the number of thirty-seven suspended between blocks of 5 per cent. gelatin, one block holding only distilled water, and the opposing one a 0.5 per cent. solution on the anhydrous nitrate of ammonium, showed when the preparations were taken down twenty-four of the roots grown into the block containing no chemical, while the other thirteen roots were straight.

A similar preparation with the same species was made with gelatin with distilled water on one side, and with gelatin and a 0.75 per cent. solution of water-free calcium nitrate on the other side. At the end of the experiment twenty roots were negative toward the salt, and the other eight roots were straight. Still another set of seedlings of the same species was prepared with roots between gelatin and distilled water on one side and gelatin and a 0.375 per cent. solution of the water-free calcium nitrate on the other. The result turned out as before, except that a smaller proportion of roots bent. Of the seventeen roots, eight

[11] ROTHERT: Beobachtungen und Betrachtungen über tactische Reizerscheinungen. Flora **88**: 409. 1901.

turned into the gelatin holding distilled water only, while nine grew straight.

Used in experiments similar to the foregoing, a 0.5 per cent. solution of potassium nitrate caused nine roots of the lupin out of ten to turn slightly into the distilled water gelatin, while the tenth root bent into the potassium nitrate gelatin. The growth was good, the roots not distorted, and the curves were slight. A 0.6 per cent. solution of anhydrous magnesium sulfate caused very slight curvatures in the lupin roots, but all ten roots certainly bent into the distilled water gelatin.

The curves described above as following the use of ammonium nitrate, calcium nitrate, potassium nitrate, and magnesium sulfate may have been either negatively traumatropic or negatively chemotropic. In view of the behavior of the roots of this plant toward gelatin containing 3.5 per cent. of cane sugar, the curves last described could not have been osmotropic. The experiments certainly do show that *Lupinus albus* is not similarly chemotropically sensitive to all salts that it may absorb as food. Its roots are positive toward all solutions of sodium phosphate tried, but they turn away from solutions of corresponding osmotic strength of the other salts used.

During the course of the work it seemed of interest to test the behavior of the roots of the lupin when two unlike salts of the same osmotic pressure were brought at the same time against opposite sides of the roots. For this purpose isosmotic solutions were made of potassium nitrate (KNO_3), calcium nitrate ($Ca(NO_3)_2$), and magnesium sulphate ($MgSO_4$). The solutions were made by the specific gravity method to give a pressure equal to 130^{cm} of mercury, it being assumed that in these weak solutions ionization was complete. It was assumed also that KNO_3 gave two ions, $Ca(NO_3)_2$ three ions, and $MgSO_4$ two ions.

When these isosmotic solutions of potassium nitrate and of calcium nitrate were opposed on the flanks of a row of lupin roots, nine of the eleven roots grew into the potassium nitrate, two roots growing straight. When potassium nitrate and magnesium sulfate were opposed, ten of the eleven roots grew into the potassium salt, and one into the magnesium. When calcium nitrate and magnesium sulfate were opposed, ten of the fourteen roots grew into the magnesium salt, while the other four roots remained neutral.

When we remember that all of the four salts last used caused the lupin roots to bend negatively when these salts were severally opposed to distilled water gelatin, we may be certain the curves noted in the preceding paragraph were repulsion and not attraction curves. The magnesium sulfate therefore repels more strongly than the potassium nitrate, and the calcium nitrate more strongly than either the potassium nitrate or the magnesium sulfate. Whether this repulsion is chemotropic or traumatropic cannot be decided at this time.

To ascertain whether other plants are chemotropically sensitive as *Lupinus albus* has been found to be, seedlings of *Cucurbita Pepo* L. have been put to the test.

Isosmotic solutions of potassium nitrate, calcium nitrate, and magnesium sulfate were prepared with computed pressures equal to 130^{cm} of mercury at a temperature of $15°$. With these solutions blocks of gelatin (6 to 10 per cent. of gelatin according to the general atmospheric temperature) were made up and brought against the roots of the seedlings suspended in rows in damp chambers. The results may be shown in tabular form, the chemicals in a horizontal row being in opposing blocks of gelatin, the column of figures to the left indicating the number of roots curving toward the salts to the left, the figures in the middle column indicating the neutral roots, and the figures at the right indicating the number of roots curving toward the salts there given.

		Curved	Neutral	Curved		
KNO_3	←	1	9	0	→	$MgSO_4$
KNO_3	"	2	16	10	"	$Ca(NO_3)_2$
$MgSO_4$	"	0	9	3	"	$Ca(NO_3)_2$

Through an error in reading the specific gravities of solutions as given in Gerlach's tables,[12] the stock solution of di-sodic phosphate was made with an osmotic pressure of 39^{cm} of mercury, instead of the same pressure as the solutions of the three salts

[12] Zeitsch. Analyt. Chemie **8**: —. 1869.

last used. Before the error was discovered, *Cucurbita Pepo* had been tested as shown in the following table, the explanation of the columns of figures being the same as for the preceding table, and the salts held in gelatin blocks as before.

		Curved	Neutral	Curved		
KNO_3	←	3	19	7	→	Na_2HPO_4
$Ca(NO_3)_2$	"	2	9	1	"	Na_2HPO_4
$MgSO_4$	"	0	8	0	"	Na_2HPO_4

It is probable that the results would have been approximately the same in the last table if the solutions of salts on the opposite sides of the roots had been osmotically equal. *Lupinus albus* was tested in precisely the same way and with the same chemicals as given in this table, and the result showed thirty roots out of thirty-seven curved toward the sodic salt, and not one root curved toward the three other chemicals.

From the two foregoing tables, this result certainly stands out: *Cucurbita Pepo* is neither attracted nor repelled by the chemicals used as is *Lupinus albus*. If its direction of growth is controlled at all, it is but feebly so by the di-sodic phosphate and the calcic nitrate; but it is more probable that it is not chemotropic, at least toward the salts here used, and that the curves that came were due to disturbances of growth, not falling within the realm of chemotropism.

CONCLUSIONS.

The experimental results recorded in the foregoing pages show that the roots of *Lupinus albus* are chemotropically positive toward solutions of di-sodic phosphate, and that no concentration of solution of this salt will produce a negative curve. The stronger solutions used (1.5 per cent.), cause first a curving toward the salt and then death. The death of the roots in such a solution may be due to the osmotic strength of the surrounding medium. The osmotic strength of the salt solution plus that of the gelatin probably amounts to somewhat more than four atmospheres of pressure, and such a pressure is probably greater than that of the cells of the distal millimeter of the root-tip.[13]

[13] STANGE: Bot. Zeit. **50**: 292. 1892.

The particular attractive component of the salt is not shown. It may be either the sodium or the phosphoric acid ion. Recalling the work of Stange[14] and of Buller[15] one might think the response to be due to the PO_4 ion. Experiments now being made in this laboratory will, it is hoped, determine this question.

The general indifference of the roots of *Cucurbita Pepo* toward the chemicals used indicates that we may expect further study of the chemotropism of roots to show the same specific differences in sensitiveness to chemicals as is shown in sensitiveness toward light; that is, there will be found chemotropic and non-chemotropic roots.

The behavior of all the roots tested gives no indication of osmotropism. *Lupinus albus* was indifferent to the one-sided application of a 3.5 per cent. solution of cane sugar, and the same plant gave no negative bends when a solution of the di-sodic phosphate concentrated enough (four atmospheres) to cause death was applied to one side only. Yet the roots of this plant are hydrotropic. The roots of *Cucurbita Pepo* were indifferent when chemicals plus the gelatin gave osmotic pressure of about 78^{cm} of the mercury on one side and 170^{cm} of mercury on the other.

From Rothert's[16] view one would regard osmotropism and hydrotropism as identical; yet it is not improbable that roots will be found which are not osmotropic though they are hydrotropic. Such is the indication in these experiments.

The behavior of the lupin roots in curving away from all chemicals used except the sodic salt, may be either traumatropic or chemotropic. Or may this not be a reaction where chemotropism and traumatropism lose their distinction?

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[14] STANGE: Bot. Zeit. **48**: 124. 1890.

[15] BULLER: Annals of Botany **14**: 558. 1900.

[16] ROTHERT: Flora **88**: 415. 1901.