"GROWTH-PROMOTING SUBSTANCE" AND ELONGATION OF ROOTS

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I

The very marked difference in geotropic response of root and of stem has been explained by implying the existence of different mechanisms, based either on the presence of starch grains in special organs (Nemec, 1900) or on the activity of growth-promoting substances formed by the tip of each organ (Cholodny, 1924).

The first tentative explanation is practically ruled out by the fact that organs of plants which have no starch-containing cells may still show definite geotropic reactions.

The second hypothesis can be considered as favoring one of two possibilities: either different substances are formed by the tips of root and stem respectively, the difference being shown by the reaction of each organ; or else the substances may be the same but elicit reactions of opposite signs by their action on different substrata.

An experiment reported by Cholodny (1924, 1926) gives a possible way of discriminating between the two views under the second hypothesis. He showed that decapitated roots of *Zea* and *Lupinus* when "tipped" again with *Avena* coleoptile tips exhibit geotropic curvature in the right direction and with normal speed, when suitably excited. In other words, substances coming from the tip of a coleoptile determine opposite movements in stem and in root, placing us therefore under the necessity of transferring to the organization of the stem or root the "choice" in the differential response.

If such a substance induces opposite responses in root and stem, one may still wonder if under geotropic excitation of stem or root differential accumulation of this growth-promoting substance takes place (1) at the lower side of the horizontal stem and at the upper side of the horizontal root, or (2) in both organs at the lower side, but with

antagonistic effects; *viz*, accelerating growth of the lower half of the stem and partially inhibiting the growth of the lower half of the root.

Cholodny (1926) has reported that the rate of growth of the roots tipped with coleoptile tips is definitely reduced.

More recently, when the experimental part of the present work was finished, a paper by Keeble, Nelson, and Snow (1931) brought out additional arguments for these views. These authors used *Zea mays* roots, which may not be the very best material for such experiments. Furthermore, the "tipping" was done in all experiments with root or coleoptile tips and no attempt was made to use the Went-Dolk technique of handling the growth-promoting substance by means of agar blocks.

II

In the course of an investigation of the mechanism of geotropic bending of roots, the rate of elongation of normal, intact roots of *Lupinus albus* was measured over durations of 2 to 5 hours while the roots were growing vertically downward, at a temperature of 22–22.5°C. Young seedlings with a root of 10 mm. in length on the average were placed on a perforated paraffin disk covering the opening of a small vial, lined almost completely with moist filter paper. Roots were in this way in an atmosphere nearly saturated with moisture. Care must be taken to remove any free drops of water (*cf.* Navez, 1933) which might accumulate at the tip of the root, either by means of a small dry brush or with a little roll of filter paper. The normal elongation was followed by means of a horizontal microscope with micrometric eyepiece. Fig. 1 shows at *A* the type of curve obtained: a straight line fits the observations over the portion of time involved in the experiment. All other conditions being the same, some roots whose normal elongation had been followed for 2 to 3 hours were decapitated at 1.0 mm. to 1.5 mm. from the tip and placed again under observation. The immediate effect of the decapitation is a complete stop in the elongation of the root, followed within 10 to 50 minutes, according to the individual seedling observed, by a renewed elongation proceeding now at a slower rate (Fig. 1, Curve *B*).

For periods of $1\frac{1}{2}$ to 2 hours this new rate remains practically constant, after which it gradually increases. The increase is obviously

due to the regenerated activity of a new "physiological tip" located at the cut section, and is analogous to that observed in the coleoptile of

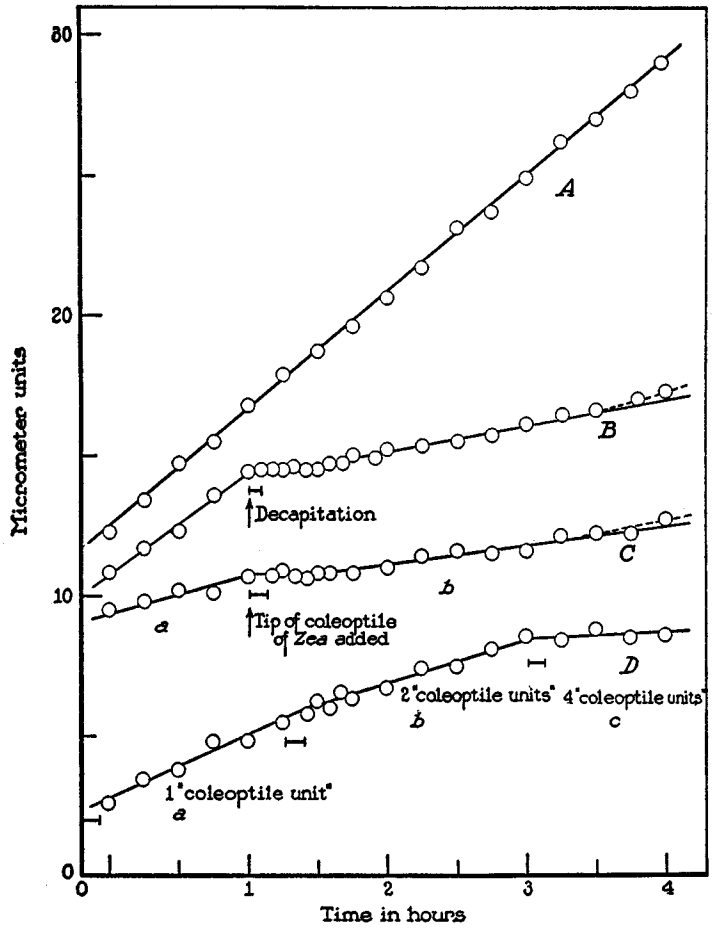

FIG. 1

Curve *A*. The normal elongation of an intact root of *Lupinus*.

Curve *B*. Elongation of a root decapitated at the moment pointed by the arrow.

Curve *C*. A decapitated root elongating at a constant rate (*a*) is provided at the moment indicated by the arrow with a tip of coleoptile of *Zea*.

Curve *D*. A decapitated root of *Lupinus* provided successively at the moments indicated by the black lines with 1, 2, and 4 "coleoptile units" of diffusate from *Avena*.

Avena by Went (1928). This physiological tip does not involve morphological regeneration, which could not take place in so short a time. The existence of the new physiological tip has been held to be demonstrated by cutting off the last millimeter of the decapitated root, which again reduces the rate of growth practically to what it was before physiological regeneration took place.

Other decapitated roots of seedlings were observed for a period of time sufficient to make sure of a definite constancy in their rate of growth (1 hour); this once established, they were tipped with tips of coleoptiles of *Zea mays* of about 1.5 mm. length, which were made adherent by touching their cut surface with a 3 per cent solution of gelatin. The adhesion of the tips is then very good, and the presence of gelatin does not of itself introduce any factor affecting growth, as control experiments have shown.

One notes in Fig. 1, Curve *C*, that after a short lag period, which may be attributed to the presence of the gelatin, a constant rate of growth is reached which is maintained for about 2 hours. Apparently the inhibition of elongation then gradually diminishes.[1] The remarkable feature of Part *b* of Curve *C* is the drop in slope. In other words, the growth substance diffusing from a coleoptile tip reduces the rate of elongation of the root.

III

To deal in a more quantitative way with the unknown growth substance we used the technique described by Went (1928), and employed *Avena* coleoptiles as the test organism to determine the concentration of active substance extracted by the agar block.[2] Agar

[1] This decrease in inhibition must be attributed to a decreased vitality of the tip of the coleoptile, either through gradual using up of its growth substance or by failure of the tip to receive the necessary materials to keep it in good condition for formation of the effective substance. It is apparently not due to internal conditions in the root, as one can keep the rate depressed by changing at regular intervals of time the tips or blocks of agar containing the diffusate from tips collected by Went's (1928) technique.

[2] Coleoptiles of *Avena* are decapitated when 25 to 30 mm. long, and tipped with agar blocks containing the diffusate of tips. The manipulations are done in the dark room at 22.0–22.5°C., the illumination used coming from a very dim red light of lower spectral limit 635 mμ. Extractions from tips by diffusion and experiments are done in a chamber the atmosphere of which is nearly saturated in water vapor. Plain agar blocks without diffusate fail to produce any effect on roots. A more complete account of certain aspects of technique will be given in a subsequent paper.

blocks 1 mm. x 1 mm. x 1.2 mm., on which tips of coleoptiles of *Zea* had been placed for 60 minutes, were divided in two halves: one served to determine the quantity of diffusate from the tip, by placing it on a prepared coleoptile of *Avena* and measuring the angle of deflection after 60 minutes; the other half was applied at the tip of a decapitated root. The time curve of elongation of a *Lupinus* root so treated shows perfect parallelism with Curve *C* of Fig. 1, lending support to the idea that the growth-promoting substance as extracted by this process is really responsible for the observed effect.

Further proof is lent by an experiment involving the use of agar blocks previously in contact with 2, 3, or 4 coleoptile tips. In such a case we get a definite increase of inhibition of elongation when we increase the amount of growth substance provided for the reaction (Fig. 1, Curve *D*).[3]

IV

The inhibition of elongation of a root by stem tip can best be proved if we provide the same root at regular intervals of time with agar blocks containing increasing amounts of growth substance from coleoptile tips. Fig. 1, Curve *D*, demonstrates this effect.

Another way of demonstrating this point is to place the block of agar in an eccentrical position, determining thus an inequal distribution of growth substance in the tissues. In such a case, a definite curvature occurs *towards* the side where the agar block is placed. One remembers that in the case of a decapitated coleoptile the same block of agar induces a curvature towards the opposite side. One can also place symmetrically on either side of a decapitated root two blocks of agar of the same dimensions, separated from one another by a small gap, one of the blocks having been in contact with two or three tips of coleoptile, the other containing only the diffusate of a single tip. In such case, the shape of the root after 3 to 5 hours of contact is definitely

[3] This result is in opposition with the observation of Cholodny (1926) who worked with hollowed-out stems of *Lupinus angustifolium*. The difference between Cholodny's results and ours may be ascribed to the type of contact prevalent in each case: a more or less loose contact between tips and receptive tissue in Cholodny's experiments, a rather perfect contact between agar block and cut section in our experiments. Moreover, the hollowing out of a stem is a more drastic treatment than the removal of the tip.

indicative of the stronger inhibition induced by the agar block containing the diffusate of two or three tips.

Some variation in the experimental results had been noticed which could only be traced to accidental shift in the position of the tips. It led us to investigate the independent reaction in growth of decapitated roots tipped with tips stimulated by gravity. For these experiments the *Zea* coleoptile tips were cut at about 2.5 mm. from the end and were used after definite periods during which they were placed horizontally in Petri dishes with their cut surfaces in contact with agar blocks. Such agar blocks were then divided into two halves corre-

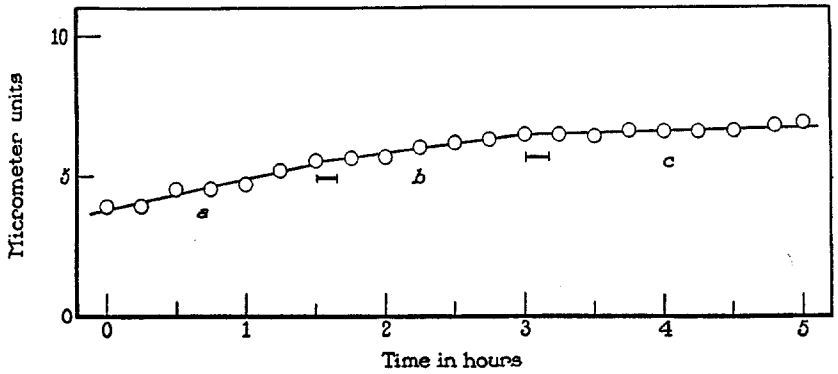

FIG. 2. A decapitated root of *Lupinus* elongating (*a*) at constant rate is provided (*b*) with the diffusate of the *upper half* of a horizontally placed coleoptile tip of *Zea*; in *c* the diffusate of the *lower half* is substituted.

sponding to the upper and lower halves of the horizontally placed tips. Each half-block was brought to act successively on the same decapitated root of *Lupinus*. In Fig. 2 the effect obtained by each block is visible: the one corresponding to the *lower* half has a much greater inhibiting effect on the elongation curve than the *upper* one.

In the case where each block of agar was placed so as to cover only one half of the cross-section, the deflection of the tip is also definitely more pronounced where the lower half block has been used, corresponding therefore to a more pronounced inhibition of growth on that side.

These experiments support the idea that there is one "growth-

promoting substance" originating in growing tips of root or coleoptile, whose action on decapitated roots results in a lower rate of elongation although the diffusate of the coleoptile accelerates the rate of elongation of the decapitated coleoptile. Experiments have been made with smaller amounts of growth substance from coleoptile tips, as determined by shorter duration of contact of decapitated tip and agar block. The inhibition is very definitely less for 30 minutes' contact; some experiments may even point to a slight acceleration for 10 minutes' contact, although we shall not stress this point before further experimentation is done.

SUMMARY

The vertical elongation of normal roots of *Lupinus* seedlings proceeds at constant rate over periods of 4 to 5 hours.

The decapitation of a root stops its elongation for a variable length of time, followed by a period of renewed elongation at a rate lower than that of the normal root.

The tipping of the decapitated root with a tip of a coleoptile of *Zea* induces a decrease in the rate of elongation of the root.

The same effect can be obtained with the diffusate from tips of coleoptile of *Avena* and to a lesser extent with diffusate of root tips.

The reduction in the rate of elongation of the root determined by diffusate from the lower half of the tip of a coleoptile placed horizontally is more pronounced than the inhibition elicited by the diffusate of the upper half of the same tip.

Various experiments with the diffusate of tips support the idea that under the conditions used the growth-promoting substance of the coleoptile tip or root tip inhibits the elongation of the decapitated root.

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