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Auxin in Coleus Stems: Limitation of Transport at Higher Concentrations

Abstract. *Previous indirect evidence that the endogenous concentration of plant hormones of the auxin type is controlled by a limitation of transport over the physiological range of concentration was confirmed by measuring directly the transport of the native auxin, indoleacetic acid, through segments of* Coleus *stems.*

Indoleacetic acid (IAA) is generally considered, on the basis of its activity and occurrence, to be the major endogenous plant hormone of the auxin type. As such, its transport properties are of particular importance in understanding plant development (*1*). Concentrations of IAA in the range of 0.1 to 10 ppm have been judged to transport approximately normal physiological amounts to plant tissue, although quantitative checks of this assumption have been rare (*2*). Indirect evidence from studies of the transport of IAA in relation to growth and regeneration of xylem supported the hypothesis that there is not a steady increase in the amount of auxin transported as the amount added is increased, but rather that a plateau is reached when the concentration is in the range of 1 to 10 ppm (*1, 3*). Earlier workers, who had tested this point directly on segments from the coleoptile of *Avena,* did not find a plateau; the more recent of them concluded that "the amount transported from apex to base (normal transport) increases almost linearly with the logarithm of the applied indoleacetic acid concentration" (*4*). However, the potential importance of such a saturation effect in the normal control of plant development is sufficient (*1, 3*) to warrant making a direct test on one of the organisms from which the indirect evidence cited was obtained. The preliminary evidence already presented was not considered conclusive, since the results might have reflected an artifact in the bioassay (*1*). The present report, therefore, describes a more detailed analysis of this system.

Studies of the basipetal translocation of auxin were made from individual segments taken from the second internode of *Coleus* plants that were closely matched for developmental age, and which were of the clone used previously. Each segment, approximately 10 mm in length, was prediffused on moist filter paper for 2½ hours, a time sufficient as shown by direct bioassay for total depletion of endogenous diffusible auxin. A 4.9-mm length was then cut from the middle of each original segment and ringed with vaseline to obviate physical movement of added IAA along the outer surface of the tissue (*3, 5*). The morphological base of each segment was placed on a washed-agar block which contained 1.5 percent plain agar and whose dimensions were 11.0 by 8.0 by 1.5 mm. A similarly prepared washed-agar block of the same dimensions, but containing IAA in concentrations of 0.5, 1, 2, 5, 10, or 20 ppm, was placed on the apical end of the segment. Auxin was usually collected for a period of 2½ hours under humid conditions at room temperature in diffuse light.

The amount of auxin in the receiver blocks placed at the base of the segment was determined on the day of, or the day after, the transport. Recovery of the amount of auxin transported was measured as the full amount and also as 1 : 2 and 1 : 4 dilutions of the full amount when the IAA had been added in concentrations of 10 and 20 ppm. Dilutions were made after the receiver block had been equilibrated for 3 hours. The block was then divided into equal halves (5.5 by 8.0 by 1.5 mm).

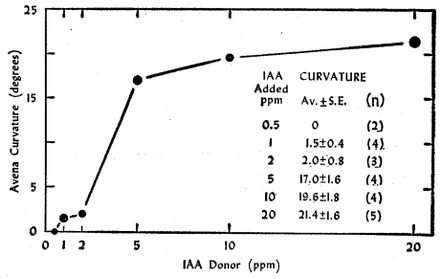

Fig. 1. Auxin recovery in undiluted, basal receiver blocks after increasing concentrations of IAA were applied to the apical ends of prediffused, 4.9-mm segments of *Coleus* stem. Transport time was 2½ hours. Values are average curvatures from bioassays (*Avena* method) of two to five separate experiments, as indicated by size of the circles and the numbers under *n*.

One of these halves was stored overnight and assayed as an index of full recovery (as compared to the assay which was made on another full-size block that had been treated similarly). The other half was stacked on a single half block of plain agar to obtain a 1:2 dilution or on a stack of three half blocks of plain agar to make a 1 : 4 dilution. The blocks were placed for 13 hours in the refrigerator in the dark in order for physical diffusion to take place. Each half block was then separated from the other and assayed independently. All blocks were assayed by the standard *Avena* curvature test (*6*). Two or more calibrations with synthetic IAA were used for each of the six tests. Results are expressed as degrees curvature.

Figure 1 shows the average curvature in the receiver blocks when the donor blocks contained various concentrations of IAA. There is a sharp increase in the amount of auxin transported as the concentration of the donor block is raised from 1 ppm to 5 ppm. An equally clear reduction in the rate of increase occurs when the concentrations of the donor block are greater than 5 ppm, although the curve does not show a flat plateau. The average curvature when the concentration of the donor block was 5 ppm was more than 1000 percent greater than when the concentration was 1 ppm, the lowest concentration resulting in transport sufficient to be detected with this method of bioassay. Yet a fourfold further increase in the concentration of the donor block (to 20 ppm) resulted in a curvature of only 26 percent more. The average curvatures from concentrations of 5, 10, and 20 ppm in the

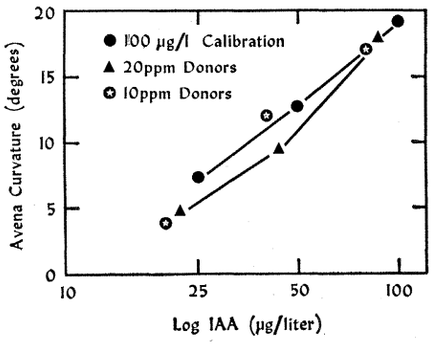

Fig. 2. Average curvatures from the *Avena* method of bioassay on undiluted, 1 : 2, and 1 : 4 dilutions of basal receiving blocks from segments treated apically with donor blocks containing 10 and 20 ppm IAA. Data from parallel dilutions of blocks containing 100 μg IAA per liter are shown as solid circles. Transport time was increased to 3 hours in order to increase the over-all yield.

donor block are not significantly different by the *t* test. Each of the six individual experiments showed a curve of the same general shape.

Since the *Avena* curvature test, which is routinely used for the bioassay of auxin, has been reported to show that higher concentration does not increase the curvature proportionately *(6)*, we must demonstrate that the receiving blocks obtained when the concentration in the donor block was 10 and 20 ppm do not contain two different concentrations of auxin both of which appear on the plateau in the bioassay *(1)*.

Parallel dilution series provide such evidence (Fig. 2). The results support the view that the donors whose concentrations were 10 or 20 ppm did not cause transport into the basal receiving blocks of amounts of auxin corresponding to those on the plateau. They also support the view that the amounts in the two types of receiver are the same, about 80 μg/liter. Finally, the agreement evident in the shapes of the response curves supports the hypothesis that the material measured in the receiving blocks is predominantly IAA. Of the 10 dilutions tried, only one showed a value that was surprising. This unexpected result occurred in a 1 : 4 dilution when the value was expected to be low.

This limitation of the amount of auxin transported at the higher concentrations of the donor block is apparently not found only in *Coleus*. A close look at the data, as contrasted to the conclusions, in some earlier investigations supports this view. For instance, a similar "plateau" effect is indicated by data on the transport of IAA through pear twigs *(7)*, and even Went's results on the *Avena* coleoptile provide—within the critical range when the concentration in the donor block is 1 to 10 ppm—evidence of a similar plateau *(4)*. Scott and Briggs have just shown that older, nonelongating portions of the pea stem transport only the normal endogenous amount of auxin even after exogenous IAA has been applied for a time sufficient to result in an abnormally high content of extractable auxin *(8)*. Recent findings indicate that a similar limitation of transport occurs in the coleoptile of *Avena* *(9)*. Recovery of C^{14} in the receiver blocks from labeled IAA in the donor blocks, approaches a maximum for 7-mm sections when the concentrations of the donor blocks are 0.8 and 1.6 ppm.

This limitation of the transport of hormone is potentially important. Results to date suggest that this limitation of transport is a controlling valve which prevents excessive production of auxin from disturbing the balanced coordination of normal development *(1)*. The available evidence though scanty also suggests that the concentration in the donor block at which the limitation begins to assert itself will vary with species; this variation is between 2 and 5 ppm for *Coleus* stems, 0.8 and 1.6 ppm for *Avena* coleoptiles *(9)*, and above 9 ppm for *Phaseolus* petioles *(10)*.

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Perception of Ultrasound

Abstract. *Ultrasonic vibrations can be perceived as audible sounds when a piezoelectric transducer is pressed against certain areas of the human body. In the range of frequencies investigated (20 to 108 kcy/sec), the threshold of perception seemed to lie near the threshold of feeling (about 10^{-4} watt/cm^2), and the perceived audible sound appeared to be between 8 and 9 kcy/sec, as judged by six test subjects. The threshold of perception and the perceived frequency appear to be dependent upon the hearing characteristics of the individual.*

As a result of an early observation by one of us (C.K.) that ultrasonic vibrations can be perceived directly as audible sounds, a series of experiments was undertaken to learn more about this effect. Piezoelectric crystals were excited into vibration at frequencies above the audible range (above 20 kcy/sec), and the vibrations were detected or "sensed" through the skin and tissue of the experimenter.

Exposure to the ultrasonic energy was achieved either by pressing a vibrating crystal directly against the body or by coupling the vibrations through a column of water placed between the crystal and the skin. The intensity of the perceived sound was dependent upon the location of the source of ultrasonic energy on the body of the observer. In the case of direct contact between the skin and a vibrating crystal, the firmness of the contact and the manner in which the crystal was held were also significant. The best sensing locations for most of the subjects were the trapezius muscle at the back of the neck, the masseter, the sternocleidomastoid, and the area of the temporal bone, particularly over the mastoid element. However, the sound was also perceived when the crystal was held at various other points, including a position near the clavicle or even lower on the chest.

Depending upon the location of the crystal and the deviation from the crystal resonance frequency, the sound appeared to originate first in one ear and then in the other, even though the location of the crystal remained the same. The frequency of the perceived sound was difficult to identify accurately. Approximate measurements were made by listening simultaneously to the "ultrasonic sound" and to the normal sound produced by a nearby loud speaker. Among the six observers, the