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Gravitropism in Higher Plant Shoots[1]

V. CHANGING SENSITIVITY TO AUXIN

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ABSTRACT

An alternative to the Cholodny-Went, auxin-transport hypothesis of gravitropic stem bending was proposed as early as 1958, suggesting that gravistimulation induces changes in sensitivity to auxin, accounting for differential growth and bending. To test the sensitivity hypothesis, we immersed marked, decapitated sunflower (*Helianthus annuus* L.) hypocotyl sections in buffered auxin solutions over a wide concentration range (0, 10^{-8} to 10^{-2} molar IAA), photographed them at half-hour intervals, analyzed the negatives with a digitizer/computer, and evaluated surface-length changes in terms of Michaelis-Menten enzyme kinetics. Bending decreases with increasing auxin concentration; above about 10^{-4} molar IAA the hypocotyls bend down; increasing auxin inhibits elongation growth of lower surfaces (which is high at zero or relatively low auxin levels) but promotes upper-surface growth (which is low at low auxin levels). Thus, lower surfaces have a greater K_m sensitivity to applied auxin than upper surfaces. At optimum auxin levels (maximum growth), growth of bottom surfaces exceeds that of top surfaces, so bottom tissues have a greater V_{max} sensitivity. V_{max} sensitivity of vertical controls is slightly lower than it is for either horizontal surface; K_m sensitivity is intermediate. Clearly, gravistimulation leads to significant changes in tissue sensitivity to applied auxin. Perhaps these changes are also important in normal gravitropism.

Upward bending of a stem placed in a horizontal position occurs because the growth rate of bottom tissues exceeds that of top tissues. Physiologists have assumed that some message, sent in response to the changed orientation of the stem with respect to gravity, must link the top and bottom stem tissues, directing this differential growth rate. For more than 60 years plant scientists have tested the hypothesis that this message was a downward transport of auxin leading to higher auxin concentrations in bottom tissues, this in turn accounting for the more rapid growth rate of those tissues (9, 27). In some systems, however, transport is not needed. In the false pulvini of grass nodes, for example, top and bottom halves can be cut apart, but auxin levels build up in the half with the epidermis facing down and decrease in the half with the epidermis facing up (30). Hence, auxin must be made or released from a bound form in the lower half and destroyed or bound in the upper half. Although no message can be transmitted in this case, it is still possible to postulate that differential growth is the result of differential auxin concentrations.

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During the past decade, this explanation of bending caused by auxin gradients has been questioned (11, 13, 16, 25). Perhaps the most important reason is that, assuming that growth rates induced by applied auxin are representative of the rates induced by endogenous auxins, measured auxin gradients seem too low to account for observed differences in growth rates. In a few cases, no auxin gradients could be detected in gravistimulated dicot stems (20) although lateral transport has often been confirmed in grass coleoptiles and in dicot stems (20, 28).

There is an alternative to the auxin-gradient hypothesis: reorientation with respect to gravity (gravistimulation) could change the sensitivity to auxin of the upper and lower tissues relative to each other and relative to vertical tissues. Even if auxin concentration does not change, if lower cells are relatively more sensitive than upper cells to the auxin already present, differential growth and upward bending would occur. Such a change in sensitivity could be in addition to or instead of changing auxin concentrations. This is presently being considered by several workers (8).

Sensitivity is the capacity of an organism or physical system (*e.g.* a microphone or a photocell) to respond to a stimulus. In this sense, sensitivity is synonymous with responsiveness. It is measured and expressed quantitatively by varying the stimulus and observing the response, that is, by obtaining a dose-response curve. Several aspects of sensitivity can be discerned from such a curve (12). When stem sections are immersed in solutions with a wide range of auxin concentrations, increasing elongation follows increasing auxin concentration (usually plotted on a logarithmic scale) until a maximum response is achieved, after which further increases in auxin lead (after some time) to decreasing response (10, 14).

Except for the supersaturation aspect of this response curve, it is convenient to think of it as being analogous to curves produced when initial reaction rates of an enzymically controlled reaction are plotted as a function of substrate concentration: the classical Michaelis-Menten kinetics (Fig. 1). The maximum reaction rate is referred to as V_{max}, and the concentration that produces a reaction rate half of V_{max} is the Michaelis constant, K_m. Thus, one kind of increasing sensitivity in the elongation response to auxin would be an increased elongation (or rate of elongation) at the concentration that produces the maximum response. In this paper, we refer to sensitivity that changes this way as V_{max} sensitivity. In most enzymically controlled reactions, V_{max} is a theoretical concept: the limit that is approached by increasing reaction rate as substrate concentration increases. The supersaturation phenomenon in the auxin response, however, makes an actual maximum response easy to determine when a sufficiently wide range of auxin concentrations has been tested. The other kind of change in sensitivity, indicated by changes in K_m, is called K_m sensitivity. An increasing value for K_m signifies decreasing K_m sensitivity. Thus, sensitivity in this system as well as in any other

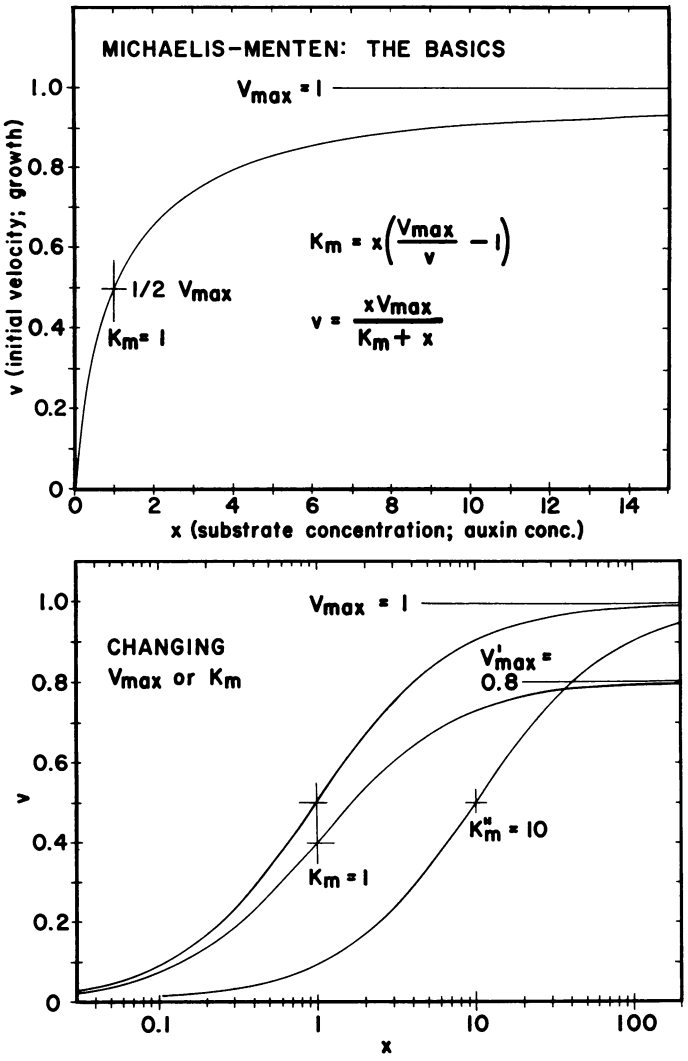

FIG. 1. The familiar Michaelis-Menten equations and curves with the definitions of V_{max} and K_m. (Top) Initial velocity of an enzymic reaction as a linear function of the substrate concentration (or, roughly, growth rate or initial growth rate as a linear function of auxin concentration). (Bottom) Initial velocity (or growth) as a logarithmic function of substrate (auxin) concentration.

system is greater if a given stimulus produces a greater response or if a smaller stimulus produces a given response.

We can imagine how sensitivity to auxin might change if we assume that auxin molecules must bind to some entity in order to cause cell elongation. V_{max} sensitivity could be determined by the number of auxin binding sites; the more active binding sites per unit amount of elongating tissue, the greater the sensitivity. K_m sensitivity could be a measure of the effectiveness of auxin binding; the more effective the binding, the greater the K_m sensitivity (and the smaller the value of K_m). We emphasize that we have no direct evidence for auxin-binding sites but only use the concept as a model to help in evaluating our results and in devising experiments.

The concept of sensitivity to auxin in gravitropism and other responses was neglected until 1981 when it was discussed by Trewavas (24, 25). Yet the basic concept was discussed by Leo Brauner and his coworkers in Munich, Germany, from 1958 until 1971, shortly before his death.

Brauner's 1958 paper, written with Hager (7), described the gravitropic memory noted already in Brauner's 1923 paper (2). Sunflower seedlings were decapitated, left for 4 d until they supposedly became depleted in auxin, and then gravistimulated in air by turning them to the horizontal. No bending occurred. After various times they were returned to the vertical and supplied with auxin (IAA) solutions in glass tubes placed on the cut surfaces. Bending (up to 40°) occurred in the expected direction (convex side had been the bottom side during gravistimulation). Brauner and Hager suggested three possible explanations for the phenomenon: some cofactor that promoted or inhibited response to auxin was transported during gravistimulation; sensitivities of the upper and lower tissues toward auxin were changed as discussed above; or permeability to the added auxin was changed unilaterally. We note a fourth possibilty: that the auxin transport mechanism was changed by gravistimulation. This appears to be Brauner's first recognition of the sensitivity hypothesis, and it may be the first mention of the hypothesis in relation to gravitropism. (Changes in sensitivity to auxin caused by aging or presence of cofactors were widely discussed in the 1930s; see Ref. 26.)

Brauner and Appel in 1960 (4) split the tip of a horizontal coleoptile in a horizontal plane and separated the halves with a mica sheet. Such a treatment stopped gravitropic bending and thus supported the concept of auxin transport. But they found that, when the split coleoptiles were immersed in an auxin solution, they bent well even when the split was horizontal. The authors concluded that, not only does tissue auxin change, but the sensitivity to auxin must change also.

Brauner and Böck in 1963 (5) studied effects of decapitation on auxin content, elongation, and the gravitropic bending capacity of sunflower hypocotyls. Two d after decapitation, the active auxin in the tissue dropped to half its initial value; after 4 d, it dropped to a third. Yet the growth capacity dropped to a fortieth after only 2 d and the ability to respond gravitropically was gone by the 4th d. To clear up this apparent discrepancy, they placed depleted hypocotyls in solutions of different auxin concentrations at different times after decapitation and measured their growth rates. The ability of the tissue to respond to auxin dropped with increasing time after decapitation, so they concluded that the drop in growth rate after decapitation is mostly caused by a drop in sensitivity rather than decreased auxin.

Hager and Schmidt in 1963 (15) confirmed auxin (labeled-IAA) transport in response to gravistimulation of *Helianthus*. They also found that isolated lower halves of auxin-depleted hypocotyls grew 42% more than those of upper halves when put in an auxin solution. They (and others, *e.g.* 1, 23, 29, 30) also found that gravitropic bending was halted in solutions of high auxin concentration although growth continued. These findings are similar to ours and will be discussed later. Both support a sensitivity mechanism, but Hager and Schmidt emphasized auxin transport and did not mention sensitivity.

The title for the English summary of Brauner's 1966 paper (3) was: "The influence of the gravitational field on the auxin-sensitivity of *Helianthus*-hypocotyls." Among other things, Brauner split gravistimulated, decapitated hypocotyls into upper and lower halves. Growth of both was promoted in 10^{-4} M IAA compared with vertical controls, but growth of lower halves was promoted more. This was evidence for changing sensitivity to auxin, and Brauner applied the explanation to the phenomenon of the gravitropic memory. The change in sensitivity developed only in oxygen, and clinostat rotation also increased sensitivity. Brauner emphasized the lateral transport of a cofactor (the message mentioned above) as the mechanism for development of sensitivity. He concluded that both auxin transport and auxin sensitivity play a role in gravitropism.

In 1971, Brauner and Diemer (6) published "The influence of the geotropic induction on the content and distribution of auxin in the hypocotyls of *Helianthus* and on their sensitivity to the growth substance." They applied auxin in agar to induce different

auxin gradients across hypocotyls and then measured bending and the auxin in the tissues. A certain measured auxin gradient in vertical hypocotyls produced a certain bending, but the same measured auxin gradient produced a much higher bending in horizontal hypocotyls (when highest concentrations were in bottom tissues). The authors concluded that sensitivity to auxin must change in response to gravistimulation. This is a simple and impressive evidence.

To the best of our knowledge, Brauner's ideas on changing sensitivity have never been quoted in reviews on gravitropism (*e.g.* 17, 21, 28). His papers are all in German, and only the last ones have English summaries. Nevertheless, the papers have been quoted in various reviews (21, 28) but never with respect to sensitivity. Hence the review here.

Wright and Rayle in 1983 (29) considered the possibility of sensitivity changes in gravitropism, finding essentially the same evidence that we will present in this paper supporting the concept (inhibition of bending by high auxin concentrations and stimulation of top growth by those concentrations). In their discussion, they reject the concept because the high auxin concentrations promoted growth of top cells—our strongest reason for supporting the idea!

We measured hypocotyl sensitivity to auxin by immersing the test sections in a wide range of auxin concentrations and measuring curvature of gravistimulated hypocotyl sections and growth of upper, lower, and vertical-control surfaces in response to these concentrations. We demonstrated a change in sensitivity to applied auxin of upper and lower surfaces compared with each other and with vertical controls. In another paper, we plan to report results of auxin measurements applied to the tissues used in our experiments and to show that applied auxin is not transported to the convex side in memory experiments (22).

MATERIALS AND METHODS

Plants and Growth Conditions. Sunflower (*Helianthus annuus* L. cv 954) seeds (kindly donated by Dahlgren & Co.[2], Crookston, MN 56716) treated with Captan/Apron fungicide were planted on a 2.5-cm grid in vermiculite, watered, and held at 34°C for 6 d (16 h light, 8 h dark), at which time the cotyledons were fully expanded, and the hypocotyls were 40 to 60 mm long. In early trials, we tested several cultivars (supplied by Berlin Nelson of North Dakota State University) in the system described below with a range of auxin concentrations. Responses of the cultivar we use are slightly more rapid and uniform than responses of other cultivars, but any sunflower cultivar gives similar results. To suggest the generality of the response, we present one set of data obtained with soybeans, *Glycine max* L. (cultivar No. A3127, Asgrow Seed Co.). Detailed results with this plant will be presented in another publication.

Standard Experiment. After several preliminary trials with various tanks and holders, based on the approach of Wright and Rayle (29) in which hypocotyl sections were immersed in auxin solutions, we developed the following system, which was used for all the experiments described in this paper:

Plexiglas tanks were constructed, 466 mm high, 600 mm wide (to match the format of a 10.2 × 12.7-cm negative), and 50 mm thick with vertical partitions that divided the volume into eight compartments, each containing a volume of about 0.7 L (vertical controls) or 0.9 L (horizontal hypocotyls) after the Plexiglas hypocotyl holders were inserted. One kind of holder, for horizontal hypocotyl sections, was a block of Plexiglas 25.4 mm thick with 10 holes drilled at equal intervals to a depth of 10 mm and a diameter of 3.0 mm, suitable for the hypocotyls. The other holder consisted of 10, 6.35-mm thick sheets of plastic cut to different lengths and cemented together to form a stairway of horizontal surfaces in which vertical holes were drilled (same dimensions). The tanks and holders can be seen in Figure 2.

At least 24 h before an experiment (to allow for temperature equilibration), 16 L of buffer (0.22 mM K_2HPO_4 adjusted with HCl to pH 6.5) were prepared with deionized water (29). Just before an experiment, indole-3-acetic acid (IAA; Sigma Chemical Co.) was dissolved in 100 mL of deionized water by adding 4 or 5 KOH pellets. After adjustment to pH 6.5, the buffer was added to make a final volume of 2.0 L and concentration of 1.0 or 0.5 × 10^{-2} M IAA. A 200-mL portion of this solution was saved and diluted with buffer for the next lowest concentration, and this was repeated to obtain the seven IAA concentrations. Buffer without auxin was used for the control.

Seedlings were pulled from the vermiculite, decapitated, and cut to a length of 30 mm. Decapitated sections bent more than intact ones and were easier to work with. They were then marked with white oil paint (titanium oxide) by rotating against two brushes in a marker we designed (Fig. 3, top). The marks, which appear as black bands on the negatives, were approximately 10 or 15 mm apart. (We tested several materials for marking including the black substances often used by others; some were unsuitable because they came off when the sections were immersed.) Holders were lined up on the bench, and marked hypocotyl sections were placed one after the other in different holders so that plants in each holder (each auxin concentration) remained about the same range of times before being immersed in the solution. That is, some plants in each holder were cut and marked almost 30 min before being immersed, while others in the same holder were immersed almost immediately; all responded about the same. During the cutting and marking process and until hypocotyls were immersed, they were kept as vertical

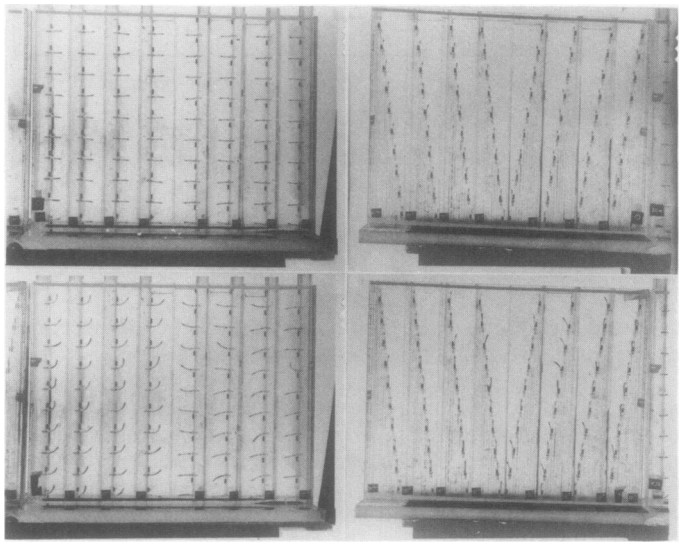

FIG. 2. Examples of photographic negatives used for analysis of growth and bending of hypocotyl sections. Marks (black) made with white oil paint are barely visible. The control (buffer) solution is on the left in each tank; the compartment next to it contains 5 × 10^{-9} M IAA in buffer, with the concentration of auxin in each compartment to the right increasing by a factor of 10. The top negatives are of horizontal and vertical sections at time zero. The bottom negatives are the same tanks 4 h later. This is the experiment of June 10, 1987, shown in Figures 4 to 7. As an example of the variability of response, note the fourth hypocotyl section from the top in the right compartment of the lower left tank. In the highest auxin concentration (5 × 10^{-3} M IAA), it is bending up while all the others bend down.

[2] Mention of trade names is to provide detailed information only and does not imply endorsement to the exclusion of other products that might also be suitable.

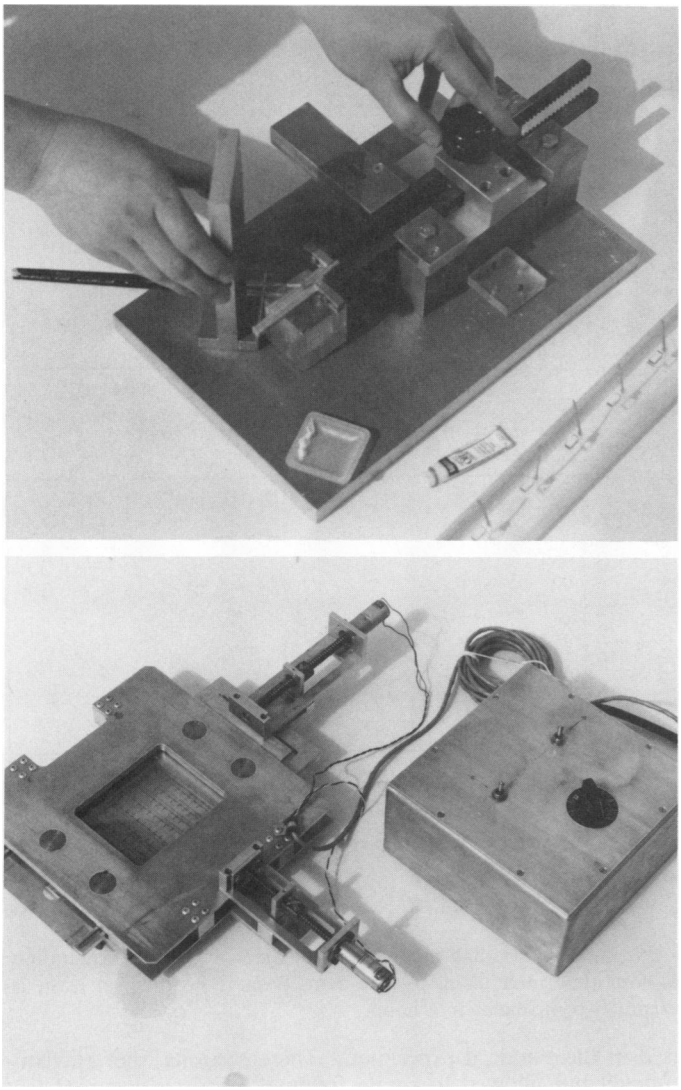

FIG. 3. Two pieces of special equipment built for the experiments described in this paper: (top) an apparatus to rotate hypocotyl sections against two brushes containing white, titanium-oxide, oil paint and (bottom) a negative carrier (left) that moves a negative in two dimensions, as controlled by the switches on the box (right). This ensures that the section being measured will always be close to the center of the optical path of the enlarger and that the section will fall on the digitizer screen.

as possible and in contact with a drop of water at the basal end. Hypocotyls were secured in the holes with 3% agar.

One batch of hypocotyls was immersed in one tank at 0 h, photographed, and then photographed at 30-min intervals. The second batch was immersed in the other tank 30 min after the first. Tanks were in a temperature-controlled (32°C) dark room in front of black velvet. Preliminary studies (see also Ref. 29) showed that plants responded nearly the same in the light or the dark whether or not solutions were aerated. Aeration sped bending slightly, but not enough to justify using it routinely; hence, it was not used.

Photographs were taken with two view cameras mounted on a rigid stand. The two matched lenses each had a 210-mm focal length, positioned 1.4 m from the tanks. We used Tri-X sheet film, ISO 320, and exposed at f/32 with an electronic flash (Honeywell Sunpack), placed to avoid shadows and reflections. Negatives were developed in Microdol-X for about 15 min at 20°C, which was overdevelopment to produce high contrast. Recently, we moved the light to produce even fewer shadows and exposed at f/45 with 10-min development. In one set of experiments, marked hypocotyl sections were floated on the buffered auxin solutions in Petri dishes as in the classical straight-growth bioassay for auxin. The Petri dishes were photographed on a light table at 30-min intervals, and the negatives were analyzed for increase in length of the hypocotyl sections.

Negatives from all experiments were projected, via two first-surface mirrors, from below onto translucent drafting vellum taped to a transparent digitizing tablet (304.8 mm square, made by Scriptel Corp., Columbus, OH). The image was 4.5 times real size when projected through a Pentax camera lens (50-mm focal length and aperture of f/1.2) to give a relatively bright image of high enlargement (25 diameters). To avoid distortions, a special negative carrier (Fig. 3, bottom) was constructed to move the negative in two dimensions (controlled by switches) so the image being measured was always close to the center of the optical path.

The software is SigmaScan 3.0 (Jandel Scientific, 65 Koch Road, Corte Madera, CA 94925). To measure stem bending, two points were digitized, the first at a surface near the apical end of the hypocotyl section and the second a short linear distance below. These data were processed through a Basic program to compute angles above or below the horizontal (stem bending in figures). The software gives lengths directly by tracing the straight or curved surfaces with the cursor. Pieces of Plexiglas 10 mm long were cemented near each hole on the hypocotyl holders, painted white for ease of measurement, and measured along with each hypocotyl to provide a calibration. The data from SigmaScan were further processed through a spread sheet (SuperCalc4) to compute actual lengths and percent length increases of the top, bottom, and vertical (control) surfaces of the hypocotyl sections.

In the basic experiment, bending and surface elongation growth were measured as a function of time and of auxin concentration. The experiment was repeated several times beginning in May of 1986. Variability in results led to many improvements in methods, but the final system as described above was used to analyze the negatives for all the experiments reported here.

RESULTS

Standard Experiment. Figure 2 shows four negatives typical of those obtained in all experiments. Figure 4 shows stem bending as a function of time for two experiments, and Figure 5 shows stem bending as a function of auxin concentration for the two experiments and for four times. Figure 5 allows us to determine the appropriate time for length measurements. Note the circa half-hour lag before bending begins (Fig. 4) and especially the typical shape of the curves in Figure 5: as IAA concentration increases above the zero (buffer) level, stem bending may or may not increase, but when moderate concentrations are reached (about 10^{-6} M IAA), bending always begins to decrease. At higher concentrations stem bending is close to zero and then negative (sections bend down; Fig. 2). There is nearly always less downward bending at the highest IAA concentrations, which are toxic (tissues visibly darkened the next day).

Figure 6 is the most meaningful plot. Data were taken at 4 h for both experiments. It is essential to compare all four curves in each figure. The stem bending curve always has the form just described, and growth of the bottom surface is always highest at the low or zero auxin concentrations, beginning to decrease with increasing IAA concentration at about 10^{-7} M IAA. Growth of the top surface is always lowest in the buffer control or in the lowest auxin concentration, increasing with increasing auxin concentration until an optimum is reached, always at a higher concentration than is optimal for the bottom surface. The increase in length at the optimum is nearly always lower for the top surface than for the bottom surface. Vertical controls often

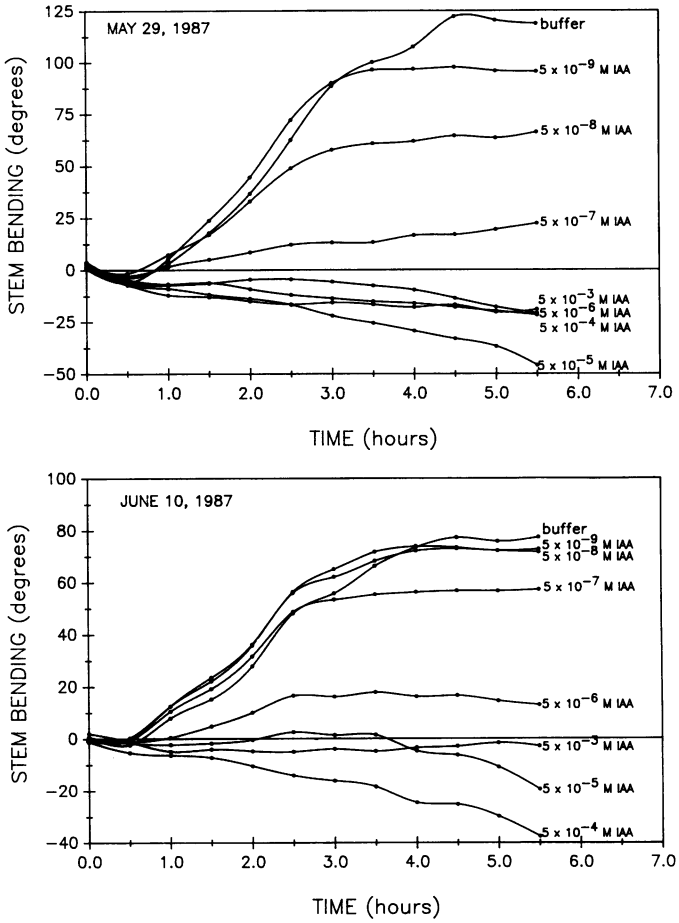

FIG. 4. Average stem bending as a function of time for the horizontal hypocotyl sections in all solutions, experiments of May 29 (top), and June 10 (bottom), 1987. Note the slight, initial downward bending and lag period that lasts about half an hour in all solutions. Note especially how sections bend less and less with increasing auxin concentrations until, at a concentration somewhere around 10^{-5} to 10^{-4} M IAA, they bend down instead of up.

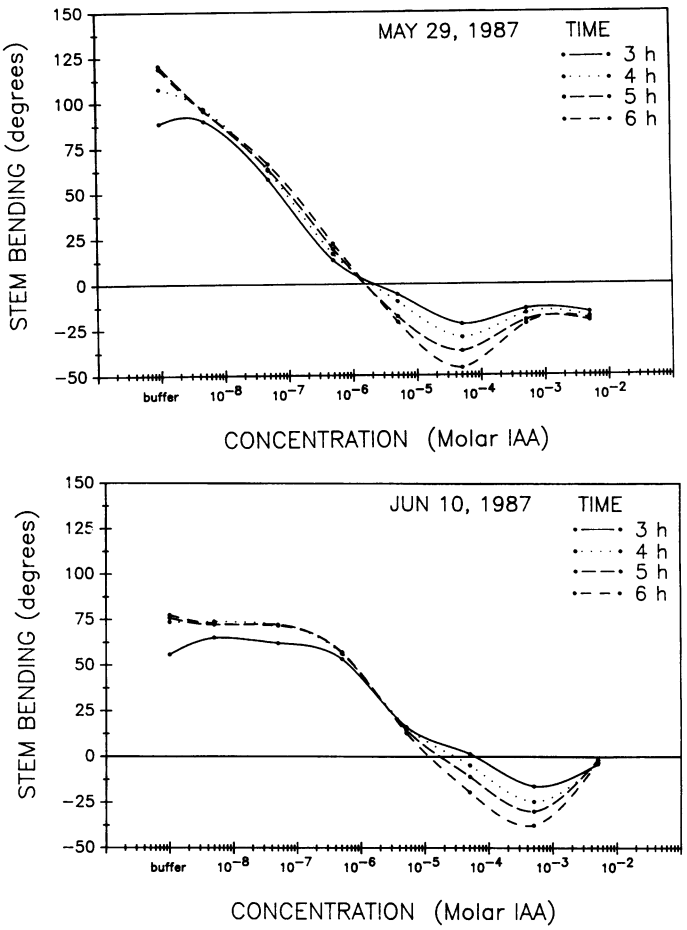

FIG. 5. Stem bending plotted as a function of auxin concentration for four time intervals after time zero. Positive bending, at least, is essentially complete by four hours.

show the expected bell-shaped curve (Fig. 6, top) for increase in length, with the maximum increase less than that for the bottom surface and often less than that for the top surface as well. The optimum IAA concentration for vertical controls in such an experiment is typically between that for the two horizontal surfaces. In some experiments, however, the vertical controls exhibit a two-peaked curve (Figs. 6, top, and 9) instead of a bell-shaped curve.

Geometrical considerations make it clear that stem bending must be predicted by the difference in growth between the top and the bottom surfaces in the region of curvature. Thus, the precision and accuracy of the length and angle measurements can be tested by comparing the difference in length between top and bottom surfaces with the stem-bending curve after adjusting the ordinate scale of the length differences so the curves will superimpose if measurements were accurate (Fig. 7). Agreement is fair to good.

Figure 8 combines stem-bending curves from six experiments. It illustrates the considerable differences between experiments.

Figure 9 shows stem-bending and growth curves for an experiment performed with soybean hypocotyls. The observations summarized above for sunflower are generally valid for soybeans.

Comparison with a Section-Growth Test. Figure 10 shows results of one experiment (typical of three) in which hypocotyl sections were floated on portions of the same auxin solutions used in the standard experiment. These sections were gravistimulated but allowed to rotate freely as bending occurred (so gravistimulation changed during the test period). Comparing the curve for the floating sections with the other growth curves, especially that for the vertical control plants, shows that the floating sections exhibited a significantly higher V_{max} sensitivity. This was extended to higher auxin concentrations than in the case of the vertical (no gravistimulation) controls.

Changing Sensitivity with Age. Figure 11 shows bending curves for hypocotyls of different ages. At any time after the beginning of the experiment, older hypocotyls have bent less, and their crossover to negative bending occurs at higher auxin concentrations. The older hypocotyls were two to three times as long as younger ones, so they had grown considerably by the time they were tested, presumably by cell elongation, but the cut sections were the same length for all ages. Thus, older hypocotyls presumably had fewer cells per section. Yet they continued to respond albeit more slowly and to a lesser degree.

DISCUSSION

Standard Experiment. The lag apparent in the low-concentration curves of Figure 4 is interesting. Is it the time required for auxin penetration or the time necessary for gravity perception and the steps that lead to the growth response to auxin? Because the lag is also apparent in the controls, auxin penetration is probably not involved. In preliminary experiments not reported here, vertical sections were pretreated for various times with auxin at the various concentrations and then turned to the

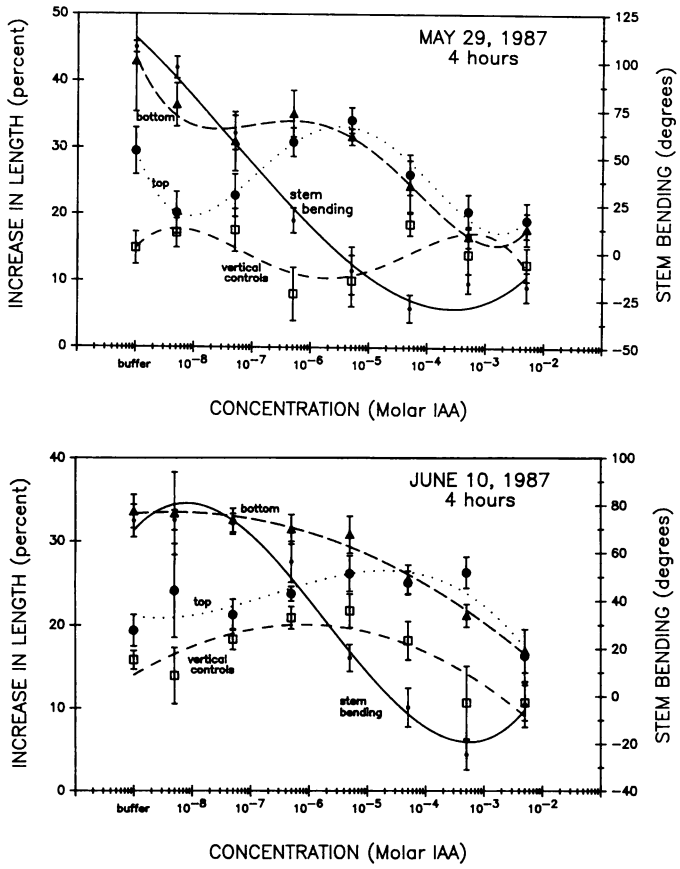

FIG. 6. Summary graph showing stem bending (angle of curvature) as a function of auxin concentration along with percent increase in distances between edges of the oil-paint marks (vertical controls plus upper and lower surfaces of gravistimulated hypocotyl sections). Bending curves are the 4-h curves of Figure 5, and all measurements were at 4 h. In all experiments of this type, lower surfaces grow much more than upper surfaces in buffer or low auxin solutions, but this is reversed at the higher auxin concentrations; this observation accounts for the bending curve. Maximum growth of the lower surface is always greater and occurs at a lower auxin concentration than maximum growth of the upper surface. Maximum growth of the vertical controls is always lower than that of either upper or lower surfaces, but it does not always produce a bell-shaped curve. There seem to be two peaks of maximum growth in the control curve of May 29th, for example, and this appears in several experiments. The curve of June 10th is nicely bell-shaped, although this is less apparent because it is plotted as a second order regression; the curves for May 29th are third order regressions (necessary to show the double peaks). Error bars represent standard error of the mean.

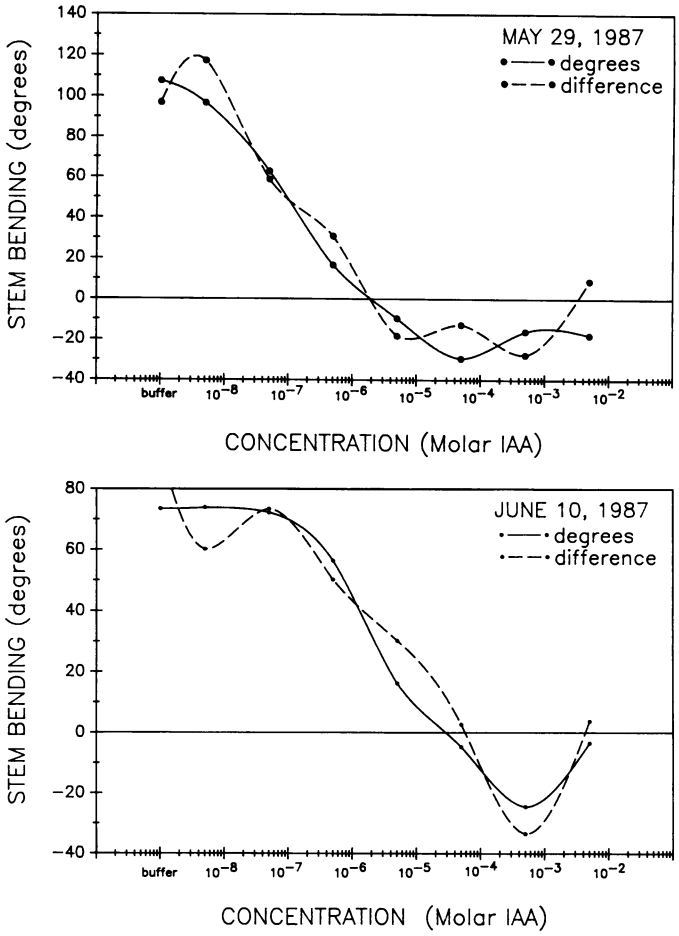

FIG. 7. Test curves to check the agreement between measured values for stem bending and the average difference between top and bottom growth. The more closely the two curves are superimposed, the more accurate were the two kinds of measurements. (The first three values on the left of the bending curve were averaged, as were the first three values for the difference between top and bottom growth. All the difference values were then multiplied by the ratio of the bending average to the difference average to adjust the difference values to match the bending scale.) Results suggest that the measurements are reasonably accurate, but there is clearly no justification for discussion of any highly subtle features of Figure 6.

horizontal. The lag was still apparent, confirming that it was not caused by auxin penetration.

Figure 6 is the key figure. As discussed in the introduction, the growth curves can be examined in light of the concepts of Michaelis-Menten kinetics to arrive at conclusions about V_{max} and K_m sensitivity. This cannot be done rigorously, however, for at least two reasons:

First, in many experiments the bottom surfaces of gravistimulated hypocotyl sections become so sensitive to auxin that V_{max} is highest in buffer (or it occurs at the lowest auxin concentrations); higher concentrations lead to inhibitions of growth. Thus, there are no points on the curve that can be used to calculate K_m (*e.g.* with the help of a Lineweaver-Burk plot; 10, 14). It can be stated, however, that K_m is some concentration less than the lowest used, and rough K_m calculations can be made for the top surface of gravistimulated hypocotyl sections; these are clearly much higher than are K_m values for bottom surfaces, showing a greatly different K_m sensitivity to auxin for the top and bottom surfaces, with the top several orders of magnitude (3 to 4?) less sensitive than bottom surfaces. If K_m sensitivity is a function of the strength of auxin binding, then binding is stronger in bottom tissues than in top tissues.

Second, the decreasing response to auxin concentrations above the optimum make application of Michaelis-Menten kinetics difficult. In a typical Michaelis-Menten plot of an enzyme reaction rate, the rate approaches V_{max} asymptotically, but the auxin curves show a negative slope after the concentration that produces maximum growth. Thus, there is no true V_{max} in the data. Nevertheless, the maximum growth is apparent, and it can be thought of as a pseudo-V_{max}. In nearly all experiments, it is lower for top surfaces than for bottom surfaces, suggesting a differential V_{max} sensitivity between the two surfaces. If V_{max} is an indication of the number of auxin binding sites, then there must be fewer in the top tissues. But there could be several factors that influence V_{max}, as Firn (12) points out. Maximum growth in the optimum auxin concentration could be limited by auxin absorption, for example, or by the capacity of the tissue to respond to the auxin-activated binding sites.

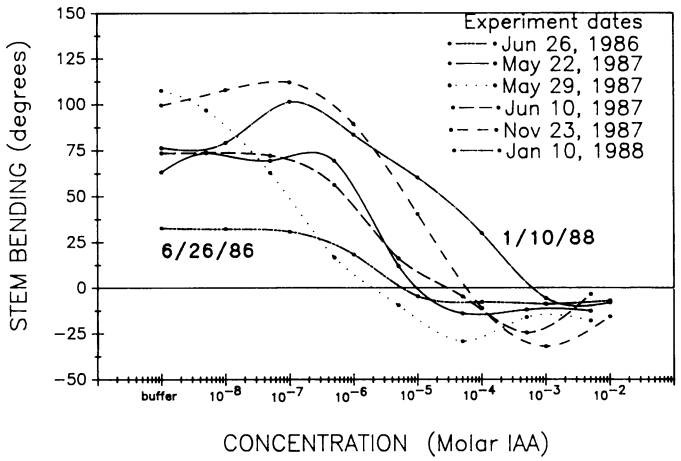

FIG. 8. Four-hour, stem bending curves for six experiments. Note the wide range of variability from one experiment to another, although all plants were grown and handled in essentially the same way (as described in "Materials and Methods"). Could some subtle environmental influence affect sensitivity to auxin and thus account for the differences? (The experiment of June 26, 1986, was a preliminary one; hypocotyls were not decapitated.)

Although a true K_m cannot be calculated for the bottom surface, the optimum concentrations (those that cause maximum growth) can be thought of as indications of a pseudo-K_m sensitivity. When this is done, it is again clear that K_m sensitivity of bottom tissues is much higher than that of top tissues, although the differences are usually less (one to two orders of magnitude) than when one visually estimates the values of K_m as the concentration that causes growth equal to one half of V_{max}.

The vertical controls often exhibit a V_{max} sensitivity below that of both the top and the bottom surfaces. Thus gravistimulation, although it may cause bending because of differential sensitivity to auxin in upper and lower tissues, apparently increases the sensitivity of both tissues. Increased sensitivity to auxin of gravistimulated tissues, both top and bottom, was also suggested by Brauner (3). When the curve for growth of vertical controls has a single peak (as in Figs. 6, bottom, and 10), the concentration at the peak typically falls between the concentrations of the peaks for top and bottom surfaces, suggesting an intermediate K_m sensitivity.

These conclusions apply without question when we speak of sensitivity to external auxin concentrations in a system such as that used in these experiments. Gravistimulation leads to greatly increased K_m and V_{max} sensitivity of bottom tissues to external auxin, and usually to an increase in V_{max} sensitivity of top tissues compared with vertical tissues. These conclusions are valid because of the way these sensitivities were defined in the introduction. The question then arises as to how these conclusions apply to normal stem tissues that are not immersed in auxin solutions. It is easy to think of reasons why they might not apply. Could increased sensitivity of lower tissues be a matter of increased penetration of auxin from the solutions into the tissues or of unilateral transport once it is inside? This can be tested by measuring the auxin in the tissues as we have been doing. So far, our measurements of uptake or transport do not account for the results presented in this paper (our unpublished data; but see Ref. 22). Could the differential sensitivity be caused by differential uptake of auxin into some subcellular compartment where it must be to promote elongation of the cells? This idea may be impossible to test because it will always be possible to postulate a mystical compartment.

Because other apparently untestable hypotheses can be suggested, it may always be impossible to prove the role of changing sensitivity to auxin in gravitropic stem bending. Yet the evidence for such a mechanism seems strong. It is difficult to account for the results presented in this paper (combined with our unpublished evidence for nearly equal uptake by both top and bottom tissues) with a hypothesis based on auxin transport or even

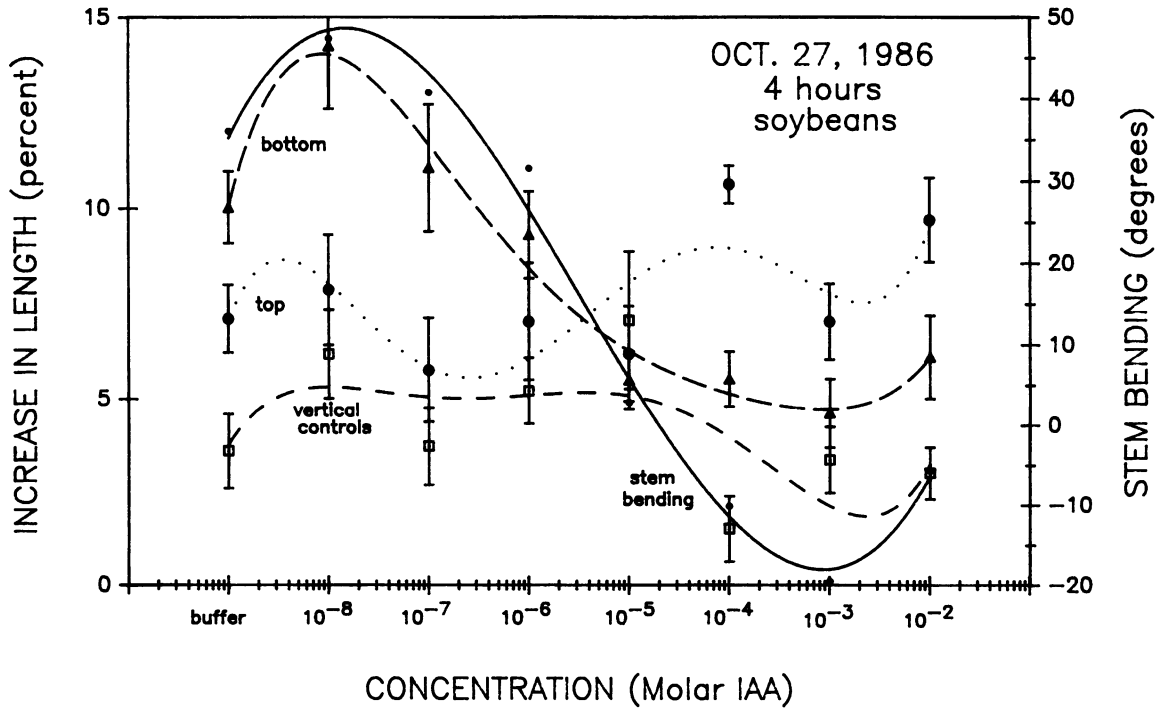

FIG. 9. Stem bending and increases in surface lengths for an experiment with soybean hypocotyls. See caption of Figure 6 for detailed explanation. Most features are similar to those observed in graphs that show results with sunflower hypocotyls. The minimal growth response of vertical controls resembles its counterpart in Figure 6, Top.

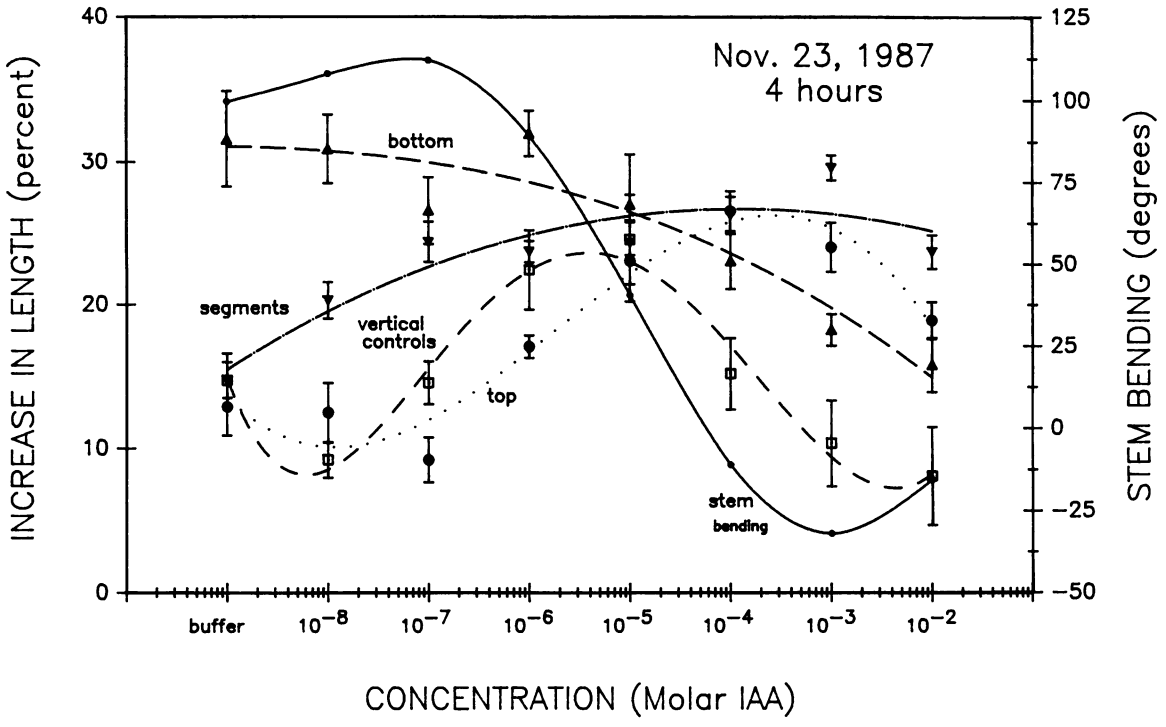

FIG. 10. An experiment with sunflower hypocotyl sections similar to those of Figure 6 except that additional sections were floated in auxin solutions in Petri dishes. Note that the sections in Petri dishes, roughly simulating the procedure followed in section-growth studies of responses to auxin, grew more than their vertical counterparts (controls). Sections in the Petri dishes were continually being gravistimulated but were free to turn as bending was initiated, so the direction of gravistimulation was changing during the 4-h growth period. Clearly, gravistimulated sections were more sensitive to auxin than vertical sections.

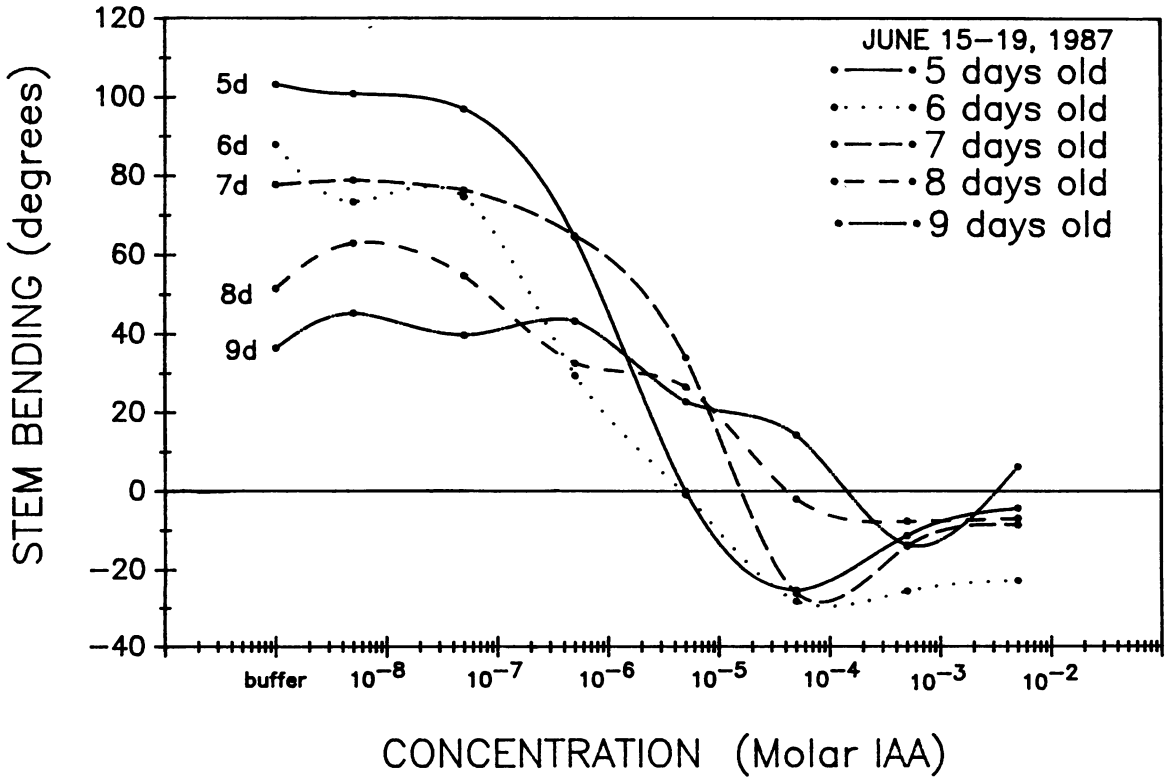

FIG. 11. Stem bending as a function of auxin concentration for hypocotyl sections of different ages but from a single batch of seedlings (June 15–20, 1987). As the hypocotyls age, they continue to grow but become less sensitive to auxin.

differential auxin synthesis and/or destruction. Nevertheless, auxin transport may play some role in gravitropic bending along with changes in sensitivity. Transport has been clearly demonstrated in many systems (reviewed in Ref. 21). It is also possible that some cofactor is transported, as Brauner and coworkers suggested (3–5, 7). The transported cofactor could produce the change in sensitivity to auxin, as brassinosteroid is known to do (19).

MacDonald and Hart (18) have proposed a mechanism in which small amounts of auxin transported downward in each half of a stem might account for bending. If one assumes that the epidermis is stimulated to grow by small increases in auxin, while the cortex below is inhibited by such increases, movement of auxin out of the upper epidermis into the cortex would inhibit growth of the upper surface, while movement out of the cortex into the lower epidermis would promote growth of the lower surface. This ingenious mechanism could play a role in gravitropic bending, but it does not explain the evidences for changing sensitivity presented in this paper.

Figure 8 emphasizes that the general shape of the bending curves of Figures 6 and 10 is dependable. That they differ greatly in detail and position almost certainly suggests that environmental conditions as well as gravistimulation can influence sensitivity to auxin.

Figure 9 is a small step in the direction of showing the generality of the results presented here. Sunflower and soybean respond to auxin in the same way.

Comparison with Section Growth Test. Figure 10 shows that hypocotyl sections floated on auxin solutions are more sensitive to auxin than are vertical sections that have not been gravistimulated. This has interesting implications for the much used section-growth test. Because the sections curve, it has long been known that they are gravistimulated. But to our knowledge, no one has considered the possibility that the results are strongly influenced by the gravistimulation, which significantly changes the sensitivity of the sections to the auxin being tested. The test may in fact be more sensitive because of the way it is carried out.

Changing Sensitivity with Age. Figure 11 suggests that sensitivity to auxin—or the sensitivity of the changing sensitivity system!—changes with age of the tissue, a phenomenon that has long been recognized (26) but not to our knowledge in studies on gravitropism.

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LITERATURE CITED

1. ANKER L 1956 The auxin concentration rule for the geotropism of *Avena* coleoptiles. Acta Bot Neerl 5: 335–341
2. BRAUNER L 1923 Über den Einfluß der Koleoptilspitze auf die geotropische Reaktion der *Avena*-Keimlinge. Ber Dtsch Bot Ges 41: 208–211
3. BRAUNER L 1966 Versuch zur Analyse der Geotropischen Perzeption V—— Ueber den Einfluss des Schwerefeldes auf die Auxinempfindlichkeit von *Helianthus*-Hypokotylen. Planta 69: 299–318
4. BRAUNER L, E APPEL 1960 Zum Problem der Wuchsstoff-Querverschiebung bei der geotropischen Induktion. Planta 55: 226–234
5. BRAUNER L, F BÖCK 1963 Versuche zur Analyse der geotropischen Perzeption IV——Untersuchungen über die Auswirkung der Dekapitierung auf den Wuchsstoffgehalt, das Längenwachstum und die geotropische Krümmungsfähigkeit von *Helianthus*-Hypokotylen. Planta 60: 109–130
6. BRAUNER L, R DIEMER 1971 Ueber den Einfluss der geotropischen Induktion auf den Wuchsstoffgehalt, die Wuchsstoffverteilung und die Wuchsstoffempfindlichkeit von *Helianthus*-Hyokotylen. Planta 97: 337–353
7. BRAUNER L, A HAGER 1958 Versuche zur Analyse der geotropischen Perzeption. Planta 51: 115–147
8. BROCK TG, PB KAUFMAN 1988 Altered growth response to exogenous auxin and gibberellic acid by gravistimulation in pulvini of *Avena sativa*. Plant Physiol 87: 130–133
9. CHOLODNY N 1926 Beiträge zur Analyse der geotropischen Reaktion. Jahrb Wiss Bot 65: 447–459
10. CLELAND RE 1972 The dosage-response curve for auxin-induced cell elongation: a reevaluation. Planta 104: 1–19
11. DIGBY J, RD FIRN 1976 A critical assessment of the Cholodny-Went theory of shoot geotropism. Curr Adv Plant Sci 8: 953–960
12. FIRN RD 1986 Growth substance sensitivity: the need for clearer ideas, precise terms, and purposeful experiments. Physiol Plant 67: 267–272
13. FIRN RD, J DIGBY 1980 The establishment of tropic curvatures in plants. Annu Rev Plant Physiol 31: 131–148
14. FOSTER RJ, DH MCRAE, J BONNER 1952 Auxin-induced growth inhibition, a natural consequence of two-point attachment. Proc Natl Acad Sci USA 38: 1014
15. HAGER A, U SCHMIDT 1963 Zum Problem der geotropischen Induktion. Ber Dtsch. Bot Ges 76: 329–341
16. HALL A, RD FIRN, J DIGBY 1980 Auxins and shoot tropisms—a tenuous connection? J Biol Educ 14: 195–199
17. JUNIPER BE 1976 Geotropism. Annu Rev Plant Physiol 27: 385–406
18. MACDONALD IR, JW HART 1987 New light on the Cholodny-Went theory. Plant Physiol 84: 568–570
19. MEUDT WJ 1987 Investigations on the mechanism of the brassinosteroid response. VI. Effect of brassinolide on gravitropism of bean hypocotyls. Plant Physiol 83: 195–198
20. PHILLIPS IDJ, W HARTUNG 1976 Longitudinal and lateral transport of [3,4-^{3}H]gibberellin A_1 and 3-indolyl (acetic acid-2-^{14}C) in upright and geotropically responding green internode segments from *Helianthus annuus*. New Phytol 76: 1–9
21. PICKARD BG 1985 Roles of hormones, protons and calcium in geotropism. *In* RP Pharis, DM Reid, eds, Hormonal Regulation of Development. III. Role of Environmental Factors. Encyclopedia Plant Physiology Spring-Verlag, Berlin, (New Series), Vol 11. pp 193–281
22. RORABAUGH PA, FB SALISBURY 1988 Measurement of auxin in gravistimulated soybean hypocotyls (Abstract 166). Plant Physiol 87: 5–28
23. THIMANN KV 1937 On the nature of inhibitions caused by auxin. Am J Bot 24: 407–412
24. TREWAVAS AJ 1982 Growth substance sensitivity: the limiting factor in plant development. Physiol Plant 55: 60–72
25. TREWAVAS AJ 1981 How do plant growth substances work? Plant Cell Environ 4: 203–228
26. WENT FW, KV THIMANN 1937 Phytohormones. Macmillan, New York
27. WENT FW 1926 On growth accelerating substances in the coleoptile of *Avena sativa*. Proc K Acad Wet 30: 10–19
28. WILKINS MB 1979 Growth-control mechanisms in gravitropism. Ch 5.2.3 *In* W Haupt, ME Feinleib, eds, Encyclopedia Plant Physiology, Vol 7, Physiology of Movements. pp 601–626
29. WRIGHT LZ, DL RAYLE 1983 Evidence for a relationship between H^+ excretion and auxin in shoot gravitropism. Plant Physiol 72: 99–104
30. WRIGHT M, DMA MOUSDALE, DJ OSBORNE 1978 Evidence for a gravity-regulated level of endogenous auxin controlling cell elongation and ethylene production during geotropic bending in grass nodes. Biochem Physiol Pflanzen 172: 581–596