Plant Physiol. (1983) 72, 99–104
0032-0889/83/72/0099/06/$00.50/0

Evidence for a Relationship between H^+ Excretion and Auxin in Shoot Gravitropism[1]

Received for publication July 6, 1982 and in revised form November 8, 1982

LUANN Z. WRIGHT AND DAVID L. RAYLE[2]
Department of Botany, San Diego State University, San Diego, California 92182

ABSTRACT

The role of auxin and protons in the gravitropic response of the sunflower (*Helianthus annuus* L. cv Sungold) hypocotyl has been investigated. No physiological asymmetry in acid-growth capacity could be detected between the upper and lower surfaces of gravistimulated hypocotyls. These data imply that neutral buffers inhibit shoot gravitropism by preventing the establishment of a lateral proton gradient along gravitropically stimulated hypocotyls. Indirect evidence that auxin is involved in the establishment and/or maintenance of such a gradient derives from the quantitative assessment of the effects of exogenous auxin, anti-auxins, and vanadate on gravicurvature. At low concentrations, exogenous auxin accelerated curvature; at high concentrations, curvature was prevented. Vanadate, an inhibitor of auxin-enhanced H^+ secretion, α-(*p*-chlorophenoxy)isobutyric acid (PCIB), an anti-auxin, and 2,3,5-triiodobenzoic acid (TIBA), an auxin-transport inhibitor, prevented observable asymmetric proton excretion using a brom cresol purple agar technique and also inhibited gravicurvature. Vanadate, PCIB, and TIBA inhibition of gravicurvature could be reversed with acid treatment to the lower surface of a gravistimulated hypocotyl. Auxin treatment to the lower surface of a gravistimulated hypocotyl did not reverse vanadate-induced inhibition, but it did partially reverse PCIB- and TIBA-induced inhibition. These results indicate a close relationship between the acid-growth theory and the differential growth responses of the sunflower hypocotyl during gravitropism.

Gravistimulation of a variety of plant shoot systems results in a fairly rapid (10–30 min) acceleration of cell extension growth on the lower side of the shoot and a retardation or cessation of cell extension on the upper side (5, 6, 12). This differential growth is evident along much of the growing axis (at least in most species), and as a result the shoot bends upward in a smooth arc (6, 15). Recently, we and others have suggested the negative gravitropic response of plant shoot systems may involve acid-induced wall loosening (3, 9, 11, 27). One line of evidence consistent with this idea is the inhibition of shoot gravitropism by neutral buffers (27).

There are at least two possible explanations for the inhibition of gravicurvature in submerged scrubbed segments by neutral buffers. One possibility is that asymmetric growth may require the development of a proton gradient. Visualization of such a gradient recently has been reported by Mulkey *et al.* (18). Such a proton gradient would lead to differential rates of acid-induced growth on the upper and lower surfaces of the organ which, in turn, would cause its gravitropic response. One could further rationalize that this proton asymmetry is possible only when the medium surrounding the segments is of low buffering capacity or when a cuticular barrier (*i.e.* nonscrubbed segments) prevents buffer penetration.

Alternatively, gravistimulation could result in a differential sensitivity of the hypocotyl cells to acid. In this case, one might imagine the normal wall pH to be slightly acidic but no physiologically meaningful asymmetry to be present. Enhanced sensitivity of cells on the lower surface to existing wall protons might then lead to differential growth. Neutral buffers in this case would act to 'stat' the wall pH at a value higher than the threshold level required to trigger the extension of the sensitized cells.

The possibility that gravistimulation may bring about a physiological asymmetry in the tissue apart from an asymmetric distribution of protons has been previously suggested by others (2, 9). For example, Ganot and Reinhold (9) reported enhanced gravicurvature in sunflower hypocotyl segments that were exposed to a gravitational stimulus while in acidic (*e.g.* pH 4.0) solutions.

The experiments presented here were designed to test the above two possibilities concerning the mechanism by which neutral buffers inhibit shoot gravitropism. In addition, data are presented which bear on the relationship between auxin and acid growth in shoot gravitropism.

MATERIALS AND METHODS

Seeds of sunflower (*Helianthus annuus* L. cv Sungold) were germinated in vermiculite and grown in growth chambers programmed for cycles of 16 h light (300 $\mu E\ m^{-2}\ s^{-1}$ at 25°C) and 8 h dark (20°C). After 4 to 5 d, seedlings were harvested, and 2.5-cm segments were cut from the region beginning just below the cotyledonary node. Preliminary experimentation showed that cotyledon and apex removal did not affect gravicurvature over the time spans utilized in these experiments. Furthermore, their removal facilitated curvature measurements.

In some experiments, in order to render the shoot more permeable to hydrogen ions and certain chemical compounds, the cuticle was gently abraded (scrubbed) by stroking (five strokes/segment) with a paste prepared by mixing water with rottenstone (finely decomposed siliceous limestone; Dowman Products, Inc., Long Beach, CA). Some studies required that only the upper or lower surfaces be scrubbed. This was accomplished by stroking the upper or lower surface of the shoot with a Q-tip dipped in rottenstone paste. Gentle abrasion of the cuticle does not appear to affect the shoot's capacity for differential growth as scrubbed and nonscrubbed segments respond similarly under optimal conditions (27). After preparation, the hypocotyl segments were rinsed with distilled H_2O, then placed in 0.25 mM K-phosphate (pH 6.8), and randomized on a rotary shaker for 30 to 45 min.

After preincubation, scrubbed and/or nonscrubbed segments were mounted in Plexiglas holders (Fig. 2). To prevent rotation or movement of the segments after insertion into the holder, their basal portions were embedded in liquid 5% (w/v) agar which

---

[1] Supported by National Aeronautics and Space Administration Grant NAGW-230.

[2] To whom requests for reprints should be addressed.

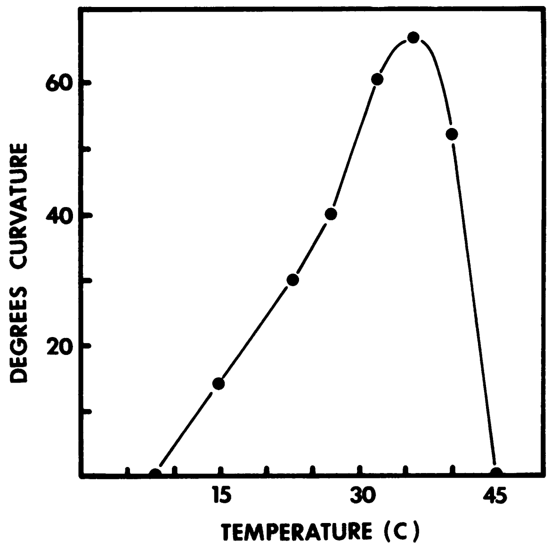

FIG. 1. Effect of temperature on the gravicurvature of nonscrubbed sunflower hypocotyl segments. Segments were submerged for 2 h in 0.25 mM K-phosphate (pH 6.8). The Q_{10} value for the temperature interval 15°C to 32°C was calculated to be 2.4.

quickly solidified. The final length of the segment which extended beyond the side of the holder was approximately 1.5 cm.

After mounting (10 segments/holder), each Plexiglas platform was submerged in a 1-L beaker containing 1,000 ml of test solution. The beaker solutions were maintained at 32°C in a water bath, a temperature found to be suitable for rapid gravicurvature of light-grown sunflower hypocotyl segments (Fig. 1). No aeration of the solution surrounding the segments was employed since experimentation indicated that aeration, under some circumstances, can retard the gravicurvature of scrubbed segments possibly by dispersal of secreted H^+ which would prevent formation of an acid asymmetry across the shoot. All manipulations prior to submerging the segments in the test solution were carried out under normal laboratory lighting. However, exposure of the segments to gravitational stimulation and their subsequent curvature occurred in the dark.

Prior to and after the appropriate period of gravistimulation, the Plexiglas holders containing the segments were photographed with a 35-mm camera. The negative showing the gravitational response was superimposed on the time-zero negative, a print made, and the amount of tip (*i.e.* upper 2 mm) displacement measured.

One technique used to test for a physiological asymmetry to acid growth employed a constant-stress extension apparatus (23). Scrubbed *Helianthus* hypocotyl sections were given 0, 30, and 60 min gravistimulation in 0.25 mM K-phosphate (pH 6.8) using the previously described submersion technique. Following gravistimulation, the shoots were bisected lengthwise, perpendicular to the force of gravity, and the halves quickly frozen. Upon thawing, the bisected hypocotyl sections were clamped in the constant-stress apparatus and subjected to 15*g* tension. The segments were then incubated in 20 mM K-phosphate (pH 6.0) until a steady extension rate was recorded. The solution was then changed to 20 mM K-acetate (pH 5.0) and acid-growth capacity of the segments was then determined by calculating the subsequent growth response in a 10-min period beginning 2 min after the buffer change.

In order to visualize hydrogen ion efflux patterns along gravistimulated shoots, a modified version of the procedure developed by Weisenseel *et al.* (26) was used. A 1% (w/v) agar solution (Bacto-Agar; Difco Laboratories) was prepared with distilled H_2O. To the liquid agar was added 0.2 g/l brom cresol purple, a pH indicator with an interval change of 5.2 to 6.8. (Below 5.2 it is yellow, above 6.8 it is purple, and between 5.2 and 6.8 it is red). Dilute HCl and KOH were used to make appropriate pH adjustments. Bright red (pH 5.8) and purple shades produced optimal results.

A 4-mm-thick plate of brom cresol purple agar was poured and allowed to cool. Scrubbed shoot segments (with and without prior gravistimulation) were embedded into the agar so that about half of their circumference was in direct contact with the agar. The plates were then oriented vertically so that the embedded shoot segments would be perpendicular to the direction of gravity. Plates were kept in the dark at 32°C. Positioning a plate in front of a fluorescent tube allowed for observation and photography. Color slides were taken using a 35-mm camera equipped with macrolens and extension tubes.

RESULTS AND DISCUSSION

To test for a possible physiological asymmetry which would render the tissue differentially sensitive to acid during gravistimulation, either the upper or lower surfaces of *Helianthus* hypocotyl segments were scrubbed before submersion in 20 mM buffer solutions at various pH. (The opposite surface retained a nonabraded cuticle.) At pH 5.0 and lower, those shoots which had been scrubbed on the lower surface responded by rapidly curving upward, while those shoots which had been scrubbed on the upper surface curved downward. These curvatures were equal in response, only opposite in direction (Figs. 2 and 3). Furthermore, when hypocotyl segments were mounted in their Plexiglas holders and given 30 min of gravistimulation in a humidifier prior to differential scrubbing and submersion, the cells on the lower surface exhibited the same degree of sensitivity to acid as those given no prior gravistimulation (Fig. 3).

Shoots scrubbed on the upper surface, however, exhibited less downward curvature when given 30 min of prior gravistimulation than did those given no prestimulation (Fig. 3). Two explanations are possible: (a) gravistimulation inhibits the acid-growth capacity of those cells on the upper surface; or (b) 30 min of gravistimulation establishes differential growth rates so that the more rapidly growing lower cells are capable of exerting greater resistance to downward acid-induced curvature.

In order to eliminate one of the above explanations as well as to provide additional data concerning a possible physiological asymmetry to exogenously applied acidic solutions, a constant-stress extension apparatus was employed to determine the intrinsic acid-growth capacity of the cells in the upper and lower halves of gravistimulated sections.

When *Helianthus* hypocotyls were given 0, 30, and 60 min of gravitational stimulation and then bisected, the lower 'half shoots' (irrespective of pretreatment time) exhibited the same intrinsic acid-growth capacity (Fig. 4). This is consistent with the previously discussed data obtained using differentially scrubbed shoot sections. One arrives, therefore, at the same conclusion using two different methods: gravitropic stimulation does not render the lower cells physiologically more responsive to acid growth. This conclusion is counter to the suggestion by Ganot and Reinhold (9) that a gravitropic stimulus may bring about a physiological asymmetry in the tissue which causes a differential response to acid buffer.

Furthermore, the upper 'half' shoots do not exhibit inhibited acid-growth capacity after gravitational stimulation (Fig. 4). On the contrary, statistical analysis indicates that after 60 min gravistimulation they have a slightly increased capacity for acid extension. While we have no explanation for this phenomenon, it does not alter our point: gravistimulation does not render the lower cells physiologically more responsive nor the upper cells less responsive to acid growth. Therefore, we conclude that the most likely explanation of the mechanism by which neutral buffers retard shoot gravicurvature is by preventing the establishment of

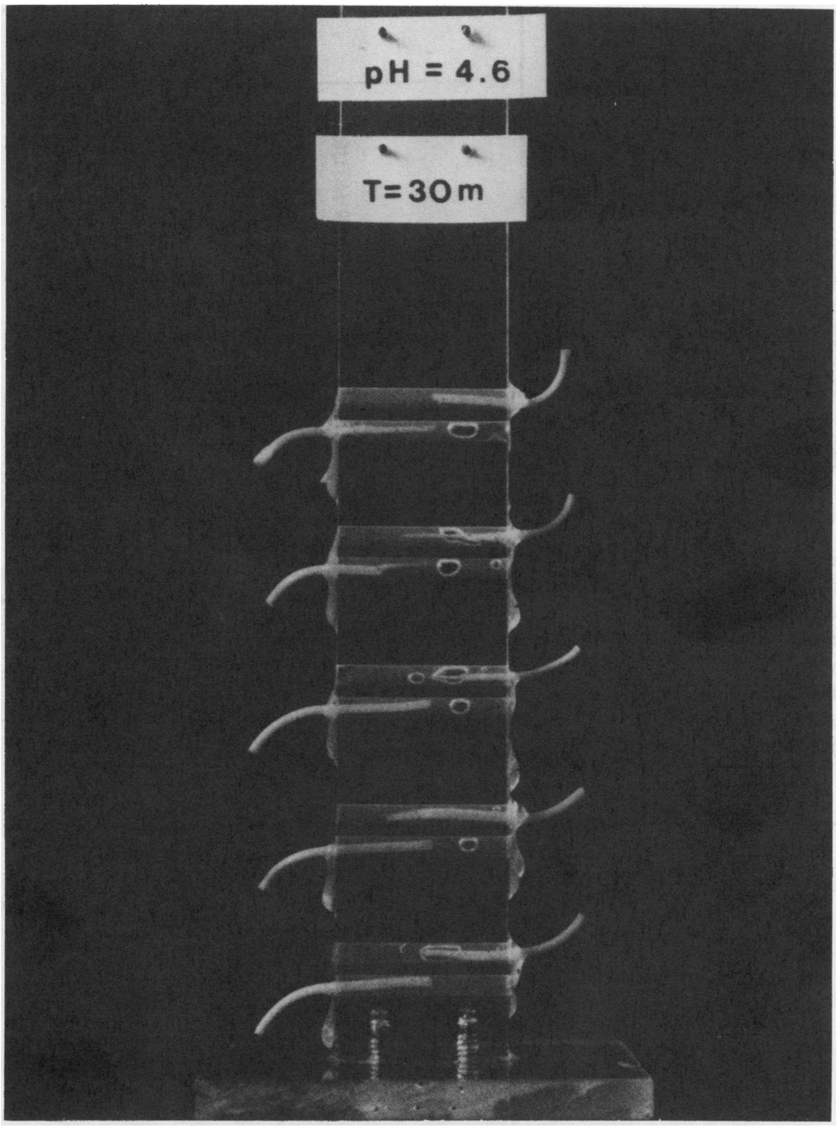

FIG. 2. Effect of pH 4.6 20 mM K-acetate on differentially scrubbed sunflower hypocotyl segments after 30 min submersion. Segments on the left were abraded on the upper surface, those on the right were abraded on their lower surface.

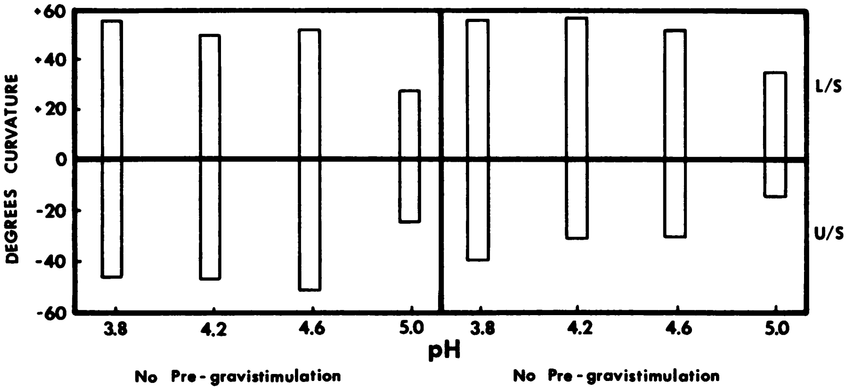

FIG. 3. Effect of 20 mM K-acetate acidic solutions on the curvature capacity of lower scrubbed (L/S) or upper scrubbed (U/S) sunflower hypocotyl segments after 30 min submersion. Shoots were given either no gravistimulation or 30 min gravistimulation prior to submersion period.

a pH gradient across gravitropically stimulated shoots.

Inasmuch as hydrogen ions are released into the wall space during auxin-induced straight growth (*e.g.* 4, 22), it is certainly possible that auxin may also play a role in the differential excretion of protons during the gravitropic response. Such a role, of course, would be consistent with the Cholodny-Went model for gravitropism. Alternatively, one could speculate that graviperception leads directly to differential proton excretion and that auxin redistribution and/or action is not critical. Such a mechanism would answer at least some of the questions recently posed by Firn and

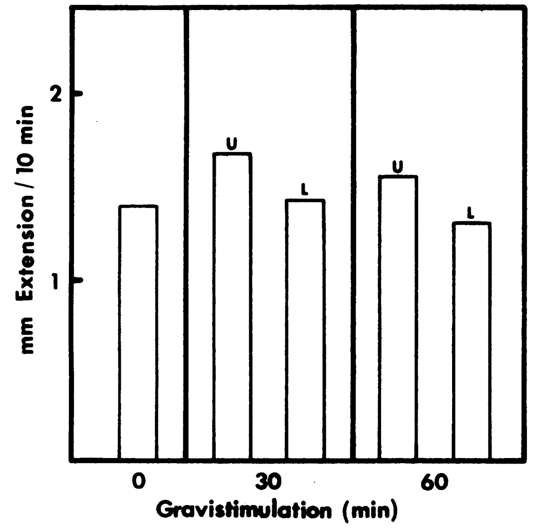

FIG. 4. Effect of 20 mM K-acetate (pH 5.0) on the extension capacity of bisected, frozen-thawed, scrubbed sunflower hypocotyls as measured on a constant-stress apparatus. Shoot segments were given 0, 30, or 60 min gravistimulation prior to bisection. (U, upper half; L, lower half). The sample sizes from left to right are 39, 37, 35, 41, 42. Statistical evaluations using a standard t test determined no significant difference ($P > 0.1$) between the upper and lower halves given 30 min gravistimulation, but a significant difference ($P < 0.05$) between the upper and lower halves given 60 min gravistimulation.

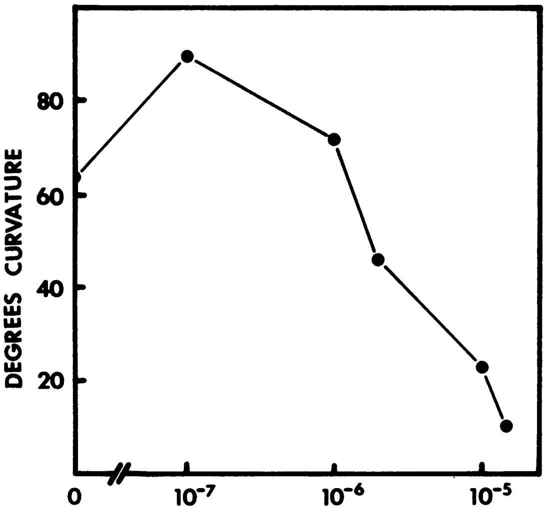

FIG. 5. Effect of various concentrations of exogenous IAA on the gravicurvature of nonscrubbed sunflower hypocotyls after 2 h submersion. Control was 0.25 mM K-phosphate. Solutions were at pH 6.8, 32°C.

Digby (8) in their criticism of the Cholodny-Went Model. We believe our technique of submerging shoots in aqueous solutions during gravitropic curvature lends itself nicely to indirect probes into the putative role of auxin in differential proton excretion and shoot gravitropism.

Our first experiment was designed to determine quantitatively the effect of exogenous auxin on shoot gravitropism. Nonscrubbed *Helianthus* hypocotyls were gravitationally stimulated while submerged in 10^{-7} to 3×10^{-5} M concentrations of IAA made up in 0.25 mM K-phosphate (pH 6.8, 32°C). Low concentrations of IAA were found to enhance gravicurvature, whereas high concentrations inhibited gravicurvature (Fig. 5). Rapid straight growth, presumably auxin induced, occurred at the higher concentrations.

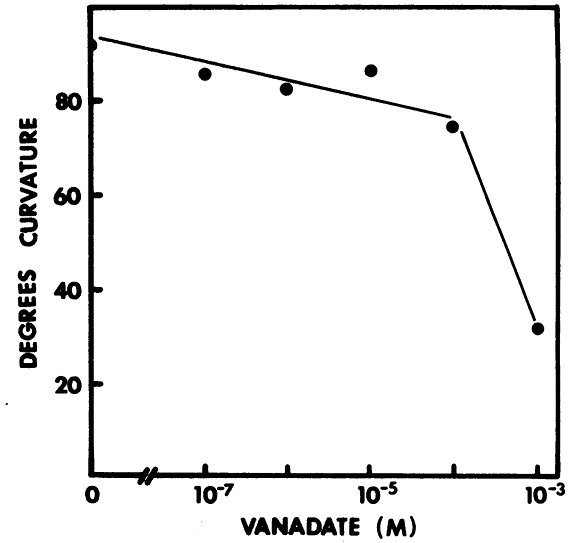

FIG. 6. Effect of various concentrations of vanadate on the gravicurvature of scrubbed sunflower hypocotyl segments after 4 h submersion. Prior to submersion, segments were pretreated for 1 h on a rotary shaker (in darkness at 32°C) with the appropriate concentration. Control was 0.25 mM K-phosphate. Solutions were at pH 6.8, 32°C.

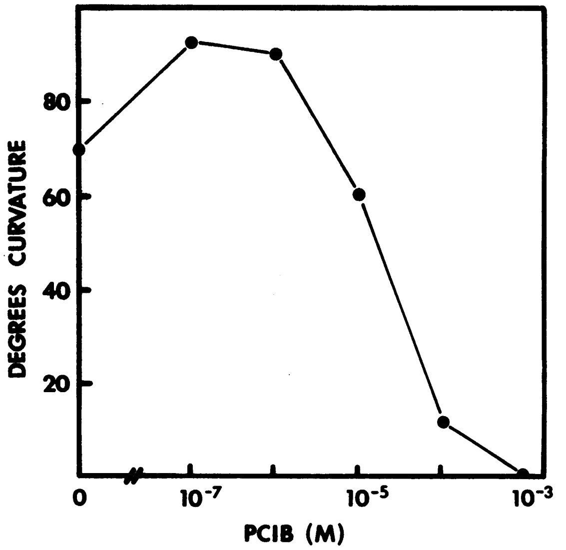

FIG. 7. Effect of various concentrations of PCIB on the gravicurvature of scrubbed sunflower hypocotyl segments after 3 h submersion. (See Fig. 6 legend for details).

Enhanced curvature at the lower concentrations was noted between 2 and 3 h after commencement of the experiments; but, after a few more hours, the control shoots displayed curvature equal to those in low molarity IAA. Low auxin concentrations, therefore, appear to speed up the gravitropic response of *Helianthus*. Similar results were obtained using *Zea* coleoptiles.

Two explanations are offered for the enhanced curvature of *Helianthus* hypocotyls in low auxin concentrations. The first possibility is that under gravistimulation the lower shoot cells are physiologically more responsive to auxin than the upper cells. One might imagine that the lower cells are stimulated during gravistimulation, by some unknown mechanism, to be more responsive to auxin. Conversely, the upper cells may be made less sensitive, perhaps due to the formation of some hypothetical inhibitor of auxin-induced growth which is activated during gravistimulation. While this explanation of physiological asymmetry cannot be eliminated, it is made somewhat less tenable by the fact that

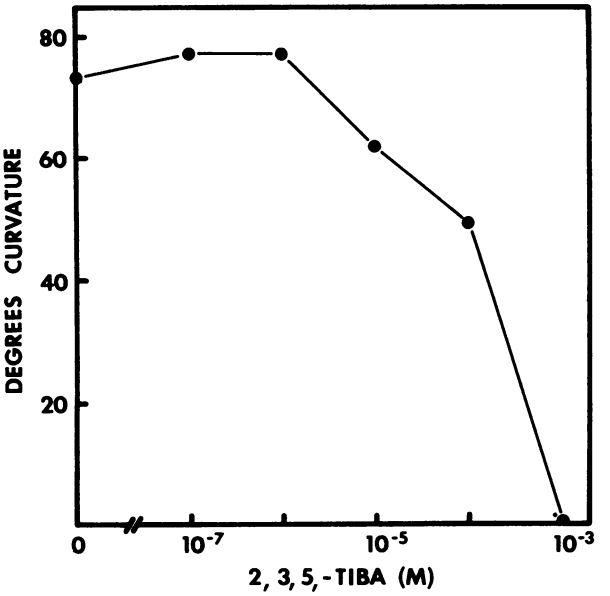

FIG. 8. Effect of various concentrations of TIBA on the gravicurvature of nonscrubbed sunflower hypocotyl segments after 2 h submersion. (See Fig. 6 legend for details).

photographic evidence confirmed that under gravitropic stimulation both the upper and lower shoots grew equally at high (*e.g.* 30 μM) concentrations of IAA which resulted in straight growth.

An alternative explanation remains. As exogenous IAA enters the shoot (presumably uniformly), one might assume that it would pool with the endogenous free auxin supply. Several investigators have reported that auxin levels increase in the lower half of a gravistimulated shoot due to lateral auxin transport (*e.g.* 7, 10, 13, 24). It is highly probable, therefore, that the additional exogenous auxin entering the lower shoot would combine with the increasing levels of endogenous auxin, raising the total auxin concentration in the lower cells to a more optimum level for auxin-induced growth. This would increase the growth rate of the lower shoot which, in turn, would create a more rapid graviresponse. The results presented here are consistent with this explanation. Furthermore, the type of gravicurvature displayed in the enhanced shoots was similar to that of the controls. The exogenous auxin, therefore, was not causing a different and/or unrelated type of curvature.

The results showing decreased curvature with higher IAA concentrations can similarly be explained. Plant cells have a maximum capacity for auxin-induced growth; hence, the lower cells will eventually reach their optimum growth rate. As the auxin levels in the upper shoots rise due to penetration of exogenous IAA, these shoots would be expected to experience increased growth. When the upper shoot cells are growing as rapidly as the lower shoot cells, straight growth would occur. Again, the results are consistent with this explanation.

Thus, auxin does appear to have an effect on gravicurvature, but whether or not it is a necessary requirement for shoot gravicurvature cannot be concluded from the above experiment. To test further the connection between auxin, proton excretion, and shoot gravitropism, sodium orthovanadate (vanadate) was used. Vanadate has been reported to be a selective inhibitor of cell membrane ion-pumping ATPases in animal (20) and fungal (1) systems. Furthermore, and more important for this study, 1 mM vanadate has been reported by Jacobs and Taiz (14) to inhibit auxin-enhanced H^+ secretion and elongation in pea epicotyls and oat coleptiles without causing either an inhibition of respiration or protein synthesis. Our preliminary experimentation confirmed that 1 mM vanadate inhibits the auxin-enhanced straight growth of *Helianthus*.

Scrubbed shoots of *Helianthus* were gravitationally stimulated while submerged in 10^{-7} to 10^{-3} M vanadate (pH 6.8, 32°C). At concentrations of 1 mM, gravicurvature was greatly inhibited (Fig. 6). These data correspond to the above-mentioned straight-growth results. Similar results were obtained using scrubbed *Avena* coleoptiles. This inhibition of curvature could be reversed with differential acid (pH 5.0) treatment but not with differential auxin (1 mM IAA in lanolin paste) treatment. (These differential treatments were administered only to the lower shoot surface.) Further, shoots pretreated in 1 mM vanadate and embedded in brom cresol purple agar exhibited no efflux of protons during gravitational stimulation. Control shoots on the other hand developed zones of H^+ efflux which were similar to those observed by Mulkey *et al.* (18) and Mulkey and Evans (16).

When scrubbed shoots of *Helianthus* were gravitationally stimulated while submerged in 10^{-7} to 10^{-3} M solutions of the antiauxin PCIB,[3] curvature was inhibited at the higher concentrations (Fig. 7). These data are consistent with those obtained by testing the effect of PCIB on auxin-enhanced straight growth of *Helianthus* (data not shown). Similar results were obtained using scrubbed *Avena* coleoptiles.

It is interesting to note that low concentrations of PCIB enhanced *Helianthus* gravicurvature (Fig. 7) as well as straight growth. Some auxin-like compounds have been reported to increase the effectiveness of low concentrations of IAA; however, no report of such synergistic activity of PCIB is known to exist. This enhancement follows the same pattern described earlier for exogenous auxin enhancement of gravicurvature at low concentrations (Fig. 5).

The polar auxin transport inhibitor TIBA was next tested in order to determine its effect on normal gravitropic activity of plant shoots. Nonscrubbed *Helianthus* hypocotyls were gravitationally stimulated while submerged in 10^{-7} to 10^{-3} M TIBA (pH 6.8, 32°C). TIBA was found to inhibit gravicurvature at the higher concentrations (Fig. 8). In addition, TIBA also inhibited auxin-enhanced straight growth. Similar results were obtained using nonscrubbed *Avena* coleoptiles.

TIBA has been established as an inhibitor of auxin transport (*e.g.* 19, 21). *In vitro* studies have shown that it competes with auxin for a membrane-bound site and that the uptake and binding of TIBA correlates with its inhibitory effect on auxin transport (25). Furthermore, an investigation by Dayanandan *et al.* (5) found that TIBA interfered with IAA- and gravity-induced growth responses in the grass pulvinus.

Both PCIB and TIBA at 1 mM were found to prevent development of a pH asymmetry along gravitropically stimulated shoots embedded in brom cresol purple agar. Mulkey *et al.* (17) previously reported that TIBA inhibits acid efflux and gravicurvature in roots. Thus, our data with shoots are consistent with their findings. Furthermore, we found the inhibitory action of both PCIB and TIBA on curvature could be circumvented by differential acid (pH 5.0) treatment and partially reversed by differential auxin (1 mM IAA in lanolin) application.

Collectively, the data presented here suggest that the lateral proton gradient which develops along gravitropically stimulated sunflower hypocotyls drives the asymmetric growth response which leads to shoot reorientation. In addition, evaluation of the effects of exogenous vanadate, PCIB, TIBA, and IAA on curvature suggests that auxin plays a role in the asymmetric extrusion of protons in this system. A similar conclusion was reached by Mulkey and Evans in experiments utilizing maize roots (16).

---

[3] Abbreviations: PCIB, α-(*p*-chlorophenoxy)isobutyric acid; TIBA, 2,-3,5-triiodobenzoic acid.

LITERATURE CITED

1. BOWMAN BJ, CW SLAYMAN 1979 The effects of vanadate on the plasma membrane ATPase of *Neurospora crassa*. J Biol Chem 254: 2928–2934
2. BRAUNER L 1969 Versuche zur Analyse der geotropischen Perzeption. V.Mitt. Über den Einfluss des Schwerefeldes auf die auxin Empfindlichkeit von *Helianthus*-Hypokotylen. Planta 69: 299–318
3. BRIDGES IG, WILKINS 1973 Acid-induced growth and the geotropic response of the wheat node. Planta 114: 331–339
4. CLELAND RE 1973 Auxin-induced hydrogen ion excretion from *Avena* coleoptiles. Proc Natl Acad Sci USA 70: 3092–3093
5. DAYANANDAN P, FV HEBARD, PB KAUFMAN 1976 Cell elongation in the grass pulvinus in response to geotropic stimulation and auxin application. Planta 131: 245–252
6. DIGBY J, RD FIRN 1979 An analysis of the changes in growth rate occurring during the initial stages of geocurvature in shoots. Plant Cell Environ 2: 145–148
7. DOLK HE 1936 Geotropism and the growth substance. Rec Trav Bot Neerl 33: 509–585
8. FIRN RD, J DIGBY 1980 The establishment of tropic curvatures in plants. Annu Rev Plant Physiol 31: 131–148
9. GANOT E, L REINHOLD 1970 The "acid growth effect" and geotropism. Planta 95: 62–71
10. GILLESPIE B, KV THIMANN 1963 Transport and distribution of auxin during tropistic response. 1. The lateral migration of auxin in geotropism. Plant Physiol 38: 214–225
11. IWAMI S, Y. MASUDA 1973 Hydrogen ion-induced curvature in cucumber hypocotyls. Plant Cell Physiol 14: 757–762
12. IWAMI S, Y MASUDA 1974 Geotropic response of cucumber hypocotyls. Plant Cell Physiol 15: 121–129
13. IWAMI S, Y MASUDA 1976 Distribution of labelled auxin in geotropically stimulated stems of cucumber and pea. Plant Cell Physiol 17: 227–237
14. JACOBS M, L TAIZ 1980 Vanadate inhibition of auxin-enhanced H^+ secretion and elongation in pea epicotyls and oat coleoptiles. Proc Natl Acad Sci USA 77: 7242–7246
15. KOHLER D 1978 The course of the orthogeotropic reactions of shoots. Z Pflanzenphysiol 87: 463–467
16. MULKEY TJ, ML EVANS. (1983) Suppression of asymmetric acid efflux and gravitropism in maize roots treated with auxin transport inhibitors or sodium orthovanadate. J Plant Growth Regul. In press
17. MULKEY TJ, KM KUZMANOFF, ML EVANS 1981 The agar-dye method for visualizing acid efflux patterns during tropistic curvatures. What's New in Plant Physiology 12(3): 9–12
18. MULKEY TJ, KM KUZMANOFF, ML EVANS 1981 Correlations between proton-efflux patterns and growth patterns during geotropism and phototropism in maize and sunflower. Planta 152: 239–241
19. NIEDERGANG E, F SKOOG 1956 Studies on polarity and auxin transport in plants. I. Modification of polarity and auxin transport by triiodobenzoic acid. Physiol Plant 9: 60–73
20. O'NEAL SG, DB RHOADS, E RACKER 1979 Vanadate inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase and other ATPases. Biochem Biophys Res Commun 89: 845–850
21. PILET PE 1965 Polar transport of radio-activity from ^{14}C-labelled-β-indolylacetic acetic acid in stems of *Lens culinaris*. Physiol Plant 18: 687–702
22. RAYLE DL 1973 Auxin-induced hydrogen ion secretion in *Avena* coleoptiles and its implications. Planta 114: 63–73
23. RAYLE DL, RE CLELAND 1972 The *in vitro* acid-growth responses: relation to *in vivo* growth responses and auxin action. Planta 104: 282–296
24. SHAW S, G GARDNER, MB WILKINS 1973 The lateral transport of IAA in intact coleoptiles of *Avena sativa* L. and *Zea mays* L. during geotropic stimulation. Planta 115: 97–111
25. THOMSON KS, R HERTEL, S MULLER, JE TAVARES 1973 1-N-naphthylphthalamic acid and 2,3,5-triiodobenzoic acid: In-vitro binding to particulate cell fractions and action on auxin transport in corn coleoptiles. Planta 109: 337–352
26. WEISENSEEL MH, A DORN, LF JAFFE 1979 Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). Plant Physiol 64: 512–518
27. WRIGHT LZ, DL RAYLE 1982 Inhibition of shoot geotropism by neutral buffers. Plant Physiol 69: 278–279