GEOTROPISM AND TRANSPORT OF INDOLEACETIC ACID IN NORMAL AND AGEOTROPIC *ZEA MAYS* L.

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

June, 1961

ACKNOWLEDGEMENTS

The author expresses appreciation to Dr. H. J. Teas for guidance in early phases of his graduate study; to Dr. A. L. Koch for assuming direction of this research in its final phases; to Dr. G. R. Noggle for providing facilities in the Department of Botany for the research; to Dr. P. H. Senn for facilitating graduate study in the Department of Agronomy; to Dr. M. J. Soule for effective criticism of this manuscript; to Drs. W. O. Ash and A. E. Brandt for advice on design and analysis of the experiments; to Dr. Poul Larsen for permission to cite from his unpublished manuscripts; to Dr. E. Horner for corn seed and facilities for field experiments; to the General Biological Supply House, Inc. for a scholarship; and to the United States Atomic Energy Commission for financial support of the research.

My wife Claire Holmsen, not only assisted measurably in many of the experiments and in preparing this manuscript but also efficiently assumed most of the responsibilities of raising our family during the writer's graduate study. Without her help and confidence this study would never have been completed.

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INTRODUCTION

Living organisms respond in a variety of ways to stimuli in their environment. Gravity, for example, has three general types of effects on plants: geotonic effect, effect of gravity on the growth rate of certain plant organs, e.g., internodes of grasses (92); geomorphic effect, effect of gravity on morphological differentiation, e.g., root formation in sugar cane cuttings (33) or flower formation in pineapple (93); and geotropic effect, effect of gravity on the orientation of plant organs. Geotropism was defined by Frank (21) as "active movement induced by gravity and oriented in a direction determined by the angle between the direction of the force of gravity and either 1) the axis of the plant part (curvature) or 2) the plane of symmetry of a bilaterally symmetrical (or dorsiventral) plant part (torsion). The direction of the movement may be that of the force of gravity (positive geotropism) or the opposite direction (negative geotropism) or the movement may take place in a plane at an angle to the direction of the force of gravity (lateral geotropism)."

Geotropic curvatures are classified according to the

liminal direction of a plant organ. The liminal geotropic direction is defined as the orientation (with respect to the plumb line) which can be maintained by a plant organ for prolonged periods of time without the organ carrying out gross geotropic reactions (51). Common types of geotropism (51, 68) are:

1. Lateral geotropism (horizontal geotropism)--the curvatures produced are in a plane at right or oblique angles to the plumb line.
2. Orthogeotropism (parallelogeotropism)--the liminal geotropic direction is parallel to the plumb line.
3. Plagiogeotropism--the liminal geotropic direction is at an angle to the plumb line.
    a. Diageotropism--liminal geotropic direction is at 90 degrees to the plumb line.
    b. Klinogeotropism--liminal geotropic direction other than parallel or perpendicular to the plumb line.

The study reported here is limited to the negative orthogeotropic reaction of Zea mays L. (corn). This plant was chosen as experimental material because of the availability of a single gene ageotropic mutant which would be expected to possess a single primary physiological deficiency.

Radioactive indole acetic acid (IAA) and recently discovered selective inhibitors of the geotropic reaction were employed in conjunction with the methods of physiological genetics to examine this important biological reaction. The radioactivity was used to measure transport and binding of the hormone; and the inhibitors used to block selectively the geotropic reaction without inhibiting growth.

The results of this study indicate that reorientation of a corn stem causes a redistribution in the physical-chemical associations of IAA within the stem. The stems of ageotropic mutant corn and stems of normal corn infiltrated with a selective inhibitor of geotropism have patterns of redistribution different from normal corn. Horizontal placement of a corn stem results in an inhibition of polar transport of IAA but has no detectable effect on lateral transport of IAA.

REVIEW OF LITERATURE

The Geotropic Reaction

Dodart (19) in 1703 referred to the propensity of plant stems to grow upward and roots to grow downward. This was apparently the first written mention of the geotropic reaction. Frank (21) coined the term "geotropism" for the "peculiar active force" liberated in plants by gravity. Many extensive reviews of geotropism, such as those by Rawitscher (68), Schrank (79), Brauner (9), and Larsen (51, 52) have been published in the past 25 years.

The geotropic reaction may be divided into three phases from an operational point of view: presentation, lag, and differential growth. According to Hawker (30), the presentation phase is the period of time plant organs must be maintained in a horizontal orientation such that upon subsequent vertical placement 75 percent of these organs develop at least 5 degrees curvature. Larsen (51) listed other definitions which have been used and presented a criticism of them. Hawker (30) examined the presentation time of a number of species of plants representing the Gymnospermae, Dicotyledonea, and Monocotyledoneae and

found times varying from 3 minutes for seedling stems of Asparagus officinalis to 24 hours for those of Phoenix dactylifera. The lag or latent phase is that period of time from the completion of the presentation phase until the observation of the first visible response (30). Hawker observed lag times of from 35 to 240 minutes. Hawker also found that presentation and lag times varied considerably with the height (age) of the seedlings tested. Prankerd (66) observed diurnal and Brain (7) seasonal variability in the duration of these phases.

The geotropic reaction may also be considered to occur in several phases from a mechanistic point of view: stimulation, transmission, and reaction (51, 68). The stimulation phase includes a physiological phase, perception or reception, and at least one physical phase, susception. Perception produces an excitation which initiates an unknown number of physiological transmission steps which culminate in the final reaction.

Two hypotheses regarding the mode of susception of geotropic stimulation have been proposed. The first and most generally accepted one states that plant organs are sensitive to differences between the liminal and actual direction of their axis (51). A more recent hypothesis

proposes that gravitational susception consists in the motion of the plant organ in moving from its normal position to a new one (6).

Regardless of the mode of susception, the effect of gravity is apparently limited to the acceleration of mass. The magnitude of this acceleration on the earth varies slightly with location but has an approximate value of 980 cm per sec^2 (g). Acceleration required to initiate the geotropic response is much less than this value. Chance and Smith (13) employing a large centrifuge determined that a resultant force between 0.019 and 0.025 g was required to elicit curvature (7.16 degrees) in seedling stems of Fagopyrum esculentum. Lyon (54), employing vibrating wires attached to an horizontal clinostat, determined that about 0.000045 g would cause bending of corn seedling roots in the dark. Experiments of Haines (27) indicated that the primary effect of gravity on plants is a redistribution of "relatively solid" particles in the protoplasts.

About 1900, Haberlandt and Nemec (26) postulated the statolith-starch theory to explain the perception of gravity by plants. According to this theory, gravity produces a displacement of starch granules which in turn excite the neighboring protoplasm. Work of many investigators, notably

Hawker (30, 31), has lent support to the theory. There are, however, many examples of plants which respond to gravity but which do not contain any starch granules (68). The existence of statoliths in plants must still be assumed in order to understand the geotropic reaction, but the morphological identity of these statoliths is unknown.

Larsen (50, 52) proposed a new model to explain geotropic stimulation as the result of extensive experiments with young roots of Artemisia absinthium (47, 49). The observed geotropic behavior of these roots fit the model of a statolith as an electrically charged pendulum with oscillations damped by a constant longitudinal force. Larsen proposed that displacement of a plant organ from its liminal direction causes a displacement of the statoliths resulting in a transverse potential which initiates the physiological processes culminating in geotropic bending. Larsen (52) developed this model mathematically and found good agreement between the model and observed geotropic behavior.

Bunning and Glatzle (11) found that presentation in two interrupted periods elicited a greater bending response than continuous presentation of the same total time. An optimum time interval of interruption was also observed. From these observations Bunning and Glatzle proposed that there

is an absolute refractory state and a relative one after geotropic irritation. According to this hypothesis, the time between successive presentations allows statoliths which were not stimulated during the first period to re-orient in a position favorable to stimulation, whereas stimulated statoliths are maintained in a temporary state of irreversible stimulation. The second period of presentation therefore allows greater stimulation, more cells are irritated, and the reaction is stronger.

Larsen (51) reviewed experiments which demonstrate the localization of cells capable of perceiving gravity. In these experiments plant organs were mounted above the axis of a centrifuge such that an extension of the axis intersected the organ at various distances from its apex. In this manner it was demonstrated that the tips of roots were far more perceptive than the region of elongation, whereas in shoots the perceptive area was more diffuse, extending into the region of elongation. These observations were confirmed by experiments in which root or shoot tips were replaced either by auxin (2) or by geotropically stimulated or nonstimulated root or shoot tips (29, 44). Further confirmation of these observations was obtained from experiments in which mica was inserted along the median plane of

horizontally placed roots for various distances from the apex before the geotropic reactivity of the roots was measured (43). These results indicate that the morphological tip of an organ, although sensitive, is not necessary for perception of gravity but functions chiefly as a source of auxin.

De Wit (18) concluded that auxin was necessary for the perception of gravity by deseeded Avena coleoptiles. This conclusion was based, among other things, on the fact that decapitated coleoptiles placed horizontally in water and then vertically in IAA solutions did not bend, whereas similar coleoptiles placed horizontally in IAA would bend when placed vertically.

It is clear, regardless of the mechanism of stimulation, that a physiological polarization has been developed at the end of geotropic presentation which leads to local differences in rates of growth. Factors examined in an attempt to characterize the nature of this polarization are: osmotic pressure, viscosity, pH, hydrolyzable sugar, reducing sugars, catalase activity, and respiration (52, 68). As Larsen (52) pointed out most of the observed changes must be regarded as a prerequisite or consequence of changes in growth rate.

Schrank (75) found that horizontal placement of an Avena coleoptile caused the lower surface to become about 10 mv more positive than the upper surface. This polarity was expressed "long before" bending or differences in auxin could be demonstrated (76). The presence of the coleoptile tip was not necessary for production of the polarity, although it was necessary for bending to occur (77). If the coleoptile was filled with an electrolyte both the geotropic bending and establishment of a potential were inhibited roughly as a function of the conductivity of the solution (78, 80). The effect of electrolytes was not osmotic (78). The establishment of this potential is the first observable effect of gravity.

Central to any explanation of the geotropic reaction is the Cholodny-Went theory. According to this theory "growth curvatures . . . are due to an unequal distribution of auxin between the two sides of the curving organ. In the tropisms induced by light and gravity the unequal distribution is brought about by a transverse polarization of the cells, which results in a lateral transport of the auxin" (95). The fact that auxin is required for the geotropic reaction is well established (2, 95). It is also well established that the lower half of a horizontally placed organ

contains more diffusible auxin than the upper half (18). Furthermore, Went (94) observed that the course of production of auxin obtained by diffusion into agar followed the course of recovery of geotropic reactivity.

However, direct confirmation of gravity-induced lateral transport has not been obtained.

In fact, recent experiments with C^{14} labeled IAA suggest that an alternative explanation is required. Bunning and co-workers (12) applied IAA 2-C^{14} to decapitated coleoptile stumps either in agar blocks or by dripping IAA solution on them. These coleoptiles were then illuminated unilaterally. After phototropic curvatures had developed, the coleoptiles were bisected. Determinations of radioactivity in the "light" and "dark" halves of over 1,000 coleoptiles failed to demonstrate any transverse transport of radioactivity. These authors also state that preliminary experiments of the same nature with the geotropic reaction failed to support the hypothesis of transverse distribution. The geotropic reaction observed could not have resulted from native auxin alone since decapitated coleoptiles require exogenous IAA for reaction (2).

Reisener (70) immersed 1.2-1.5 cm coleoptile tips vertically in solutions of 1 mg per liter radioactive IAA. The

coleoptiles were bisected three hours after horizontal placement. Again, the radioactivity in each half of the geotropically bent coleoptiles was equivalent; i.e., no evidence of lateral transport of the radioactivity was obtained.

Ching and Fang (14) applied carboxyl labeled IAA-C^{14} to pea, lima bean, and corn roots and shoots and then determined the radioactivity in the upper and lower halves of horizontally placed organs. Geotropic bending was observed in some of the organs assayed since samples were taken at intervals of 30 to 180 minutes after horizontal placement. And again, no unequal distribution of radioactivity was observed. Five to 10 percent of the recoverable radioactivity was found chromatographically identical with IAA.

An alternative to lateral redistribution of auxin has been proposed in the case of certain plant organs. The geotropic behavior of rhizomes of Aegopodium podagraria (5) and of roots of Pisum sativum seedlings (3, 4) suggested that the geotropic reaction results from the de novo production of an inhibitor rather than the redistribution of auxin.

The final geotropic reaction results from unequal growth of the upper and lower halves of a horizontally placed organ. It is well established for many species that

cell elongation is intimately involved in this differential growth (51). Brandes and McGuire (8) found that cell division as well as cell elongation contributed to the geotropic bending of sugar cane stems.

Chemical Inhibition of Geotropism

Many growth regulators have been found to inhibit the geotropic response. Some of these growth regulators inhibit both straight growth and geotropic bending, others inhibit geotropic bending but not straight growth, and still others inhibit straight growth but not geotropic bending (Table 1). The existence of these three classes of compounds indicates that the mechanism of the differential growth phase of the geotropic reaction is not identical with the mechanism of straight growth. For example, compounds which inhibit straight growth but not differential growth must act on loci of the straight growth process which do not exist in the differential growth process.

The effects of two of the compounds, 2,3,6-trichlorobenzoic acid (TCBA) and N-1-naphthylphthalamic acid (NP), have been studied extensively. Vander Beek (87) found that TCBA inhibited the geotropic response of seedling shoots of oat, barley, and cucumber. Jones and co-workers (40) reported that TCBA inhibited both geotropic and phototropic

Table 1.--COMPOUNDS WHICH INHIBIT STRAIGHT GROWTH AND/OR GEOTROPIC BENDING*

Compound	Plants	Organ	Author	Remarks
A. Compounds which inhibit straight growth and geotropic bending				
1. Ethionine	*Avena*	coleoptile	81	10 mg per liter
2. 1-naphthoxyacetic acid	rice	roots	71	10^{-4} M
3. Barium p-chlorophenyl-nitramine and analogs	rye-grass and rape	roots	41	10-50 ppm
4. Phthalamic and benzoic acid derivatives	rye-grass and rape	roots	40	
B. Compounds which inhibit straight growth but not geotropic bending				
1. 3,4-dichlorophenyl-nitramine and analogs	rye-grass and rape	roots	41	1-10 ppm
2. Phthalamic and benzoic acid derivatives	rape	roots	40	10 ppm
C. Compounds which inhibit geotropic bending but not straight growth				
1. 2,3,6-trichlorobenzoate	*Avena*	coleoptile	82	10^{-4}, 10^{-3} M
	wheat	roots	45	10^{-5} M
2. 2,4,6-trichlorophenoxy-acetic acid	*Phleum pratense*	roots	10	25 mg per liter
3. 2,4-dichlorophenoxy-acetic acid	*Avena*	coleoptile	32	1,000 ppm
4. Indolebutyric acid	wheat	leaf sheath	55	100 ppm
5. 2,3,5-triiodobenzoic acid	*Avena*	coleoptile	87	10^{-5}, 10^{-4} M
	wheat	roots	45	10^{-6}, 10^{-5} M

*Compounds which inhibit geotropic bending (42, 73) but which have not been tested in the same system for effects on straight growth have not been included.

responses of rye-grass roots without inhibiting straight growth. Recently, Schrank (82) showed that concentrations of 10^{-5} to 10^{-3} M TCBA stimulated the growth of both 5 mm subapical and 15 mm apical coleoptile sections. This stimulation was obtained only in the absence of IAA. At concentrations of 10^{-4} and 10^{-3} M TCBA inhibited both geotropic and phototropic bending of 15 mm apical coleoptile segments. Growth of these segments at 4^{o} C was neither stimulated nor inhibited by 10^{-4} M TCBA, whereas the geotropic reaction was measurably inhibited. Schrank interpreted these results as indicating that TCBA in some way inhibits the geotropic perception mechanism.

NP has been observed to inhibit the geotropic reaction of radicles of Lens esculenta, Pisum sativum, several of the Cruciferae and Compositae (57), inflorescence stems of Antirrhinum majus (86) and roots and stems of many other monocotyledonous and dicotyledonous species (59). Jones, et al (40) found that low concentrations of NP inhibited the geotropic response of rape and rye-grass roots without inhibiting root growth. Ching, et al (15) observed that NP inhibited the geotropic response of Avena coleoptiles, coleoptiles and roots of corn, and roots and shoots of Pisum sativum. Certain concentrations of NP inhibited the

geotropic reaction of these test organs with only a slight inhibition of straight growth.

An extensive study of the relationship of chemical structure to activity in inhibiting the geotropic reaction was made by Mentzer and co-workers (56). Twenty-five compounds with structures related to NP were assayed for activity in inhibiting the geotropic response of Lens esculenta seedling roots. These authors deduced the chemical structure necessary for activity (Diagram 1). Activity

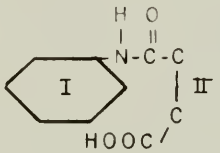


DIAGRAM I

was enhanced: (1) if the ring (I) was multiple, (2) if the carbon-carbon bond (II) was unsaturated, or (3) if the carbon-carbon bond (II) was associated with a ring system. Jones, et al (40) employing rye-grass and rape seedling roots found that the peptide linkage was not necessary for activity in inhibiting the geotropic response (e.g. 2-carboxybiphenylamine).

Jones and co-workers (40) also found that NP was neither an antagonist nor synergist of IAA in the split pea test and concluded that the antigeotropic activity of NP results neither from auxin nor anti-auxin activity. Ching, et al (15) could not "unambiguously" classify NP either as a

growth-promoting substance or a competitive inhibitor of IAA on the basis of Avena section tests. In contrast to these results, Morgan and Soding (58) found that 1 to 100 mg per liter solutions of NP promoted growth of 3 mm Avena sections floating on the solutions. Growth promotion by NP occurred both in the absence and presence of exogenous IAA (0.1 to 1.0 mg per liter). However, in assay methods requiring polar transport NP inhibited growth both in the presence and absence of IAA. Morgan and Soding (58) concluded that although NP stimulates growth it is an inhibitor of polar transport of auxin.

Auxin Transport

The role of auxin transport in the geotropic reaction is implicit in the Cholodny-Went theory. Furthermore, the physical separation of regions of geotropic perception and reaction suggests the involvement of transport processes.

Recent investigations of auxin transport have been reviewed by Leopold (53) and Van Overbeek (91); earlier studies on polar, i.e., basipetal, transport are summarized by Went and Thimann (95). Polar transport is a metabolic process having a Q_{10} of about three. The rate of polar transport is greater than accounted for by diffusion, being about 1 to 1.5 cm per hour. At 0° C the polarity of the process is maintained, but the rate of transport approaches that of diffusion. In low concentrations of ether vapor the

polarity of the process is reversibly suspended, and the rate of transport approaches that of diffusion. Wickson and Thimann (96) found that older stem segments transported less auxin than younger ones and that both light and kinetin reduced the rate of polar transport. The rate of polar transport is also proportional to oxygen tension in the range of 0 to 5 percent oxygen (25).

The transport process in Avena coleoptiles is strictly polar, that is, no acropetal transport is observed (95). More recent studies indicate that in other plant tissues this strict polarity does not hold (53). For example, Wickson and Thimann (96) were able to demonstrate measurable acropetal transport of IAA-C^{14} in pea stem sections.

Polar transport of IAA is affected by a number of compounds which also inhibit geotropic bending to a greater extent than straight growth. Among these are: TCBA (46); NP (58); 2,3,5-triiodobenzoic acid (60, 62); 2,4,6-trichlorophenoxyacetic acid (61); 2,4-dichlorophenoxyacetic acid (61); and 2,6-dichlorobenzoic acid (46). Niedergang-Kamien and Leopold (61) pointed out the similarity between the effects of eleven chlorinated phenoxyacetic acids on IAA transport and their adsorption onto charcoal. This fact, in addition to others, suggested to them that the inhibition of polar transport by these compounds might result from

interference at some transport site of attachment.

The literature contains little information on the effect of gravity on polar transport. Vander Weij (88) reported that polar transport in inverted Avena coleoptile sections was slightly inhibited. In a subsequent study Pfaeltzer (64) found no effect of gravity, acting along the longitudinal axis of the plant, on polar transport of auxin in Avena coleoptiles.

Direct studies on lateral transport of auxin in plants have likewise been neglected. However, since unilateral application of auxin to plants produces bending, the rate of lateral transport must be low. Regarding lateral transport in general, Zimmermann (98) states that "lateral transport in the phloem is known to be very slight." In support of this statement he cites several examples in which unilateral defoliation produces asymmetric growth of stems, flowers, and fruit.

Auxin Uptake

Studies of auxin uptake not only provide information on transport processes, but also on physical-chemical associations of auxin in cells. Reinhold (69) was able to distinguish two phases of uptake of IAA by pea epicotyl segments and carrot root disks; a metabolic phase and a

physical phase. Uptake by the metabolic process was inhibited by cyanide, iodoacetate, and arsenite and depressed by diethyldithiocarbamate and 2,4-dichlorophenoxyacetic acid (2,4-D). The IAA taken into the tissue by the metabolic process was not recoverable but was either bound, converted, or destroyed. Uptake by the physical process resembled adsorption rather than diffusion. Uptake by this process accounted for about 50 percent of total uptake. The IAA taken up by the physical process was essentially recoverable from the tissue.

Johnson and Bonner (37) were able to distinguish three kinds of uptake of 2,4-D into Avena coleoptile sections: metabolic uptake, diffusion, and exchangeable binding. The phase of metabolic uptake had the same properties as found for IAA (69). The diffusion phase was complete in 30 minutes and the 2,4-D taken up was free to diffuse out again into water. The inward diffusion was not influenced by 1,000-fold excesses of IAA. Exchangeable binding within the tissue was also complete within 30 minutes. The 2,4-D taken up by exchange could not be recovered into water but was released into either 2,4-D or IAA solutions. Exchangeable binding also differed from the diffusion process in that excess IAA suppressed exchangeable binding.

Recently Andreae and Ysselstein (1) found that pea roots accumulated IAA to a much greater extent than did epicotyls. During the first two to four hours of uptake IAA was recoverable from the tissue in the free form. After this period IAA was rapidly conjugated to indoleacetylaspartic acid. No other conjugated form of IAA was recoverable from the tissue during the 24-hour period of their experiments. They also found evidence that degradation of IAA occurred in only a small area of the tissue, probably the epidermis and root cap.

Active Transport vs. Permeation

Collander (16) defined "permeation" as the transfer process "in which the protoplast plays the passive role of a mere resistance to be overcome by the substance as it leaves or enters the cell." He listed six criteria to distinguish between permeation and active transport: (1) Generally the rate of a permeation process is proportional to concentration, whereas in active transport this proportionality is not found. (2) Chemically similar substances rarely compete with one another in simple permeation, but often mutually depress the uptake of one another by active transport. (3) The permeation power of substances is correlated with their molecular size and lipid solubility. If the uptake of two

substances of similar molecular size and lipid solubilities is markedly different then the uptake of at least one of them is probably not due to permeation alone. (4) Permeation is little affected by the absence of oxygen, whereas anaerobiosis, in aerobic organisms, either depresses or prevents active transport. (5) The effects of narcotics on permeation are complex, while active transport may be reversibly reduced by narcosis. (6) "Substances known to inhibit certain enzymes, such as hydrocyanic acid, carbon monoxide, sodium azide, dinitrophenol, iodoacetate, and fluoride, have been successfully used to show that particular enzymes are involved, directly or indirectly, in certain absorption processes. A pronounced effect of enzyme inhibitors on permeation processes, although conceivable, is not very probable" (16).

Collander (16) pointed out that accumulation of a substance within a cell does not necessarily imply an active metabolic transport of that substance. For example, many weak bases enter cells by permeation process, but accumulate within the cells as a result of binding. Similarly, diffusible ions may accumulate within cells as the result of Donnan equilibrium.

In the case of simple diffusion Fick's law states that

$$\frac{dQ}{dt} = -Da \frac{dC}{dx} ;$$

where dQ is the quantity of a substance which in time, dt, passes across an area, a, in which dC/dx is the concentration gradient. The constant of proportionality, D, is the diffusion coefficient with the dimensions of area divided by time, e.g., square centimeters per second (34). Larsen (48) tabulated the results of several determinations of the diffusion coefficient of IAA; D was found to be in the range of 0.596 to 0.677 cm^2 per day or an average value of about 7.45×10^{-6} cm^2 per second at 25° C.

If the cell membrane is a barrier to diffusion then obviously Fick's law does not hold for the permeation process. In this case, Fick's law may be modified to define a permeation constant instead of a diffusion constant (16). The permeation constant of IAA has not been determined, however, since IAA may be taken up or bound in several ways.

Disposition of Auxin in Tissue

It is apparent that the auxin available for transport, diffusible auxin, is only a fraction of the total auxin content. The term "diffusible" is not necessarily descriptive of the mechanism of the transport process itself. The auxin remaining in the tissue after diffusion into agar blocks is

called "bound" auxin. Roughly 50 percent of the auxin in a plant is bound (53). The identity of the molecules to which auxin is bound and the nature of the binding is not certain. Several kinds of auxin-protein complexes have been found (24, 84, 97). The possibility remains, however, that these complexes resulted as an artifact of preparation (23). Recently, Galston and Kaur (22) found evidence of auxin binding to protein in the supernatant of homogenized-centrifuged pea stem sections but could find no auxin associated with cell particulates. They also found an auxin-induced decrease in the heat coagulability of cytoplasmic protein. In a review, Galston and Purves (23) cite the work of M. Bach and also of V. Freed which also showed auxin-induced changes in the heat coagulability of cytoplasmic protein. V. Freed also found altered infrared spectra for enzyme-auxin complexes.

Lazy Corn--An Ageotropic Mutant

The literature contains a number of references to plant parts which normally change their response to gravity during some phase of their life cycle. Striking examples of such plant parts are the stamens of Hosta caerulea (65) and the inflorescences of water hyacinth (63). Of equal physiological interest are genetic mutants which fail to respond

to gravity. Such ageotropic mutants are known in rice (38, 67), Cajanus cajan (17), Pisum sativum (74), and in corn.

The ageotropic mutant of corn, discovered in 1923, was named "lazy" by Jenkins and Gerhardt (36). In field plantings lazy corn usually cannot be distinguished from normal corn until the plants enter the phase of rapid elongation just prior to tasseling. At this time the stems of lazy plants gradually bend until the stalk above the fifth or sixth node above the prop roots rests on the ground (Figure 1). After becoming prostrate the lazy plants continue to grow above the ground. Analysis of the breeding behavior of over 4,000 plants in F_2, F_3, and backcross generations indicated that the lazy character is inherited as a simple Mendelian recessive (36).

Jenkins and Gerhardt (36) undertook a detailed comparison of the characteristics of lazy and normal corn in an attempt to elucidate the action of the lazy gene. In this comparison they employed sibling plants from a backcross of homozygous lazy (la la) with heterozygous normal (La la) F_1 plants. They found that: (1) Lazy and normal plants had similar morphological structure. (2) The cell walls of lazy plant stems were thinner than those of normal plant stems.

Fig. 1.--Third generation sibling lazy (left) and normal (right) corn plants grown in the greenhouse.

(3) The breaking strength of mature green lazy plant stems was about 50 percent of the breaking strength of normal plants. (4) Lazy plant stems were lower in cellulose, lignin, and pentosans than were normal stems. (5) The expressed sap of lazy plant stems contained less ash, total solids, and ionizable constituents and had lower osmotic pressure than the sap of normal stems. Jenkins and Gerhardt (36) concluded that the lazy habit of growth resulted from a structural weakness in the stem of lazy corn.

Van Overbeek (89) found that five-to six-day-old lazy corn seedlings were negatively geotropic in the dark. However, ten-day-old seedlings in the greenhouse appeared ageotropic since they continued to grow in the direction in which they were pointed for some weeks. Furthermore, both lazy and normal plants grew parallel to the axis of a horizontal clinostat (one-half rpm) for one month. These results suggested to Van Overbeek (89) that the lazy habit of growth resulted from a deficiency in the geotropic reaction rather than a structural deficiency.

Van Overbeek (90) compared the auxin content of normal and lazy corn plants segregating from a backcross of homozygous lazy (la la) with heterozygous normal (La la) F_1 plants. Auxin was obtained by short-term cold ether

extraction and determined by Avena bioassay. Van Overbeek determined that the "nodes" (one-fourth inch on either side of the point of leaf insertion) of horizontally placed normal corn stems contained less auxin than the nodes of lazy corn stems: 0.197 ± 0.054 micrograms IAA equivalents per kilogram fresh weight for normal as opposed to 0.322 ± 0.042 for lazy. Both types of plants had equivalent amounts of auxin in internodal tissue.

The relative distribution of auxin in the upper and lower halves of normal corn stem nodes and internodes was in agreement with previous findings with other plants (95). If the upper half of the stem is taken to have 100 parts of auxin, then the lower half of nodal tissue of normal corn stems was found to contain an average of 121 parts (range 84-144) and internodal tissue 107 parts (range 85-134). In contrast, the lower half of lazy corn stem nodes contained an average of 90 parts auxin (range 67-109) and the lower half of internodal tissue 87 parts auxin (range 44-114). From these data Van Overbeek (90) concluded that the lazy habit of growth resulted from an impairment of gravity-induced lateral transport of auxin.

Van Overbeek (90) observed three classes of lazy plants in field plantings: plants lying flat with tips

"more or less" curved up, plants lying flat and "entirely straight," and plants "more or less curved downward." If plants of this last class were rotated 180 degrees along their longitudinal axis, the tip of the stem bent down again after ten days.

Shafer (83) determined that the growth of both normal and lazy corn stems occurred in regions one to four mm above the leaf insertion points of the leaves. In agreement with previous work (95) he found that horizontal placement of normal corn stems stimulated growth at nodes which would not ordinarily have grown more. Geotropic bending of normal corn stems was accompanied by the formation of a visible wedge of tissue in the region of the growth ring; the internodal regions remained straight (Figure 2). Horizontal placement of lazy corn stems elicited neither growth nor geotropic responses. Similarly the application of 0.2 percent heteroauxin in lanolin produced no growth response.

Shafer (83) compared auxin production in normal and lazy sibling plants from a backcross, i.e., no inbreeding was employed. Shafer obtained auxin from coleoptile tips of both lazy and normal seedlings (classified by subsequent growth) and then assayed the auxin by the standard Avena test. He could find no difference in auxin production

Fig. 2.--The geotropic reaction in normal corn stems results from growth in an area just distal to the nodes. The lower leaves of this plant were removed to show the new wedge of tissue formed in this growth. Note that the internodal regions remain straight. The plant was photographed two days after horizontal placement.

between normal and lazy coleoptile tips. Shafer stated that this result was expected since young lazy seedlings are negatively geotropic.

Shafer (83) also studied auxin transport in segments of the growing regions of normal and lazy stems. One cm segments of stem were clamped in a horizontal position and the morphological base of the segment divided into two equal parts by the horizontal insertion of a piece of razor blade. Plain agar platelets were placed in contact with the end of the segment, above and below the razor blade and an agar platelet containing auxin placed on the morphologically upper end of the segment. The concentration of the auxin was "varied in the direction that seemed to promise the best transport results." Shafer did not specify either the concentration of auxin used or the time interval for transport. Further, it is not clear what "auxin" Shafer was talking about.

In normal stem segments more auxin was found in the lower agar platelet on six independent trials, more in the upper twice, and essentially the same amount in each platelet nine times. The ratio of auxin in the lower platelets to that in the upper was three to two. In lazy stem segments more auxin was found in the lower agar platelet on

five independent trials, more in the upper one seven times, and the same amount in each platelet five times. The ratio of auxin in the lower platelet to that in the upper was about four to five. On the average, lazy stem segments transported more auxin than normal. The results were quite variable and the basis for comparison of normal and lazy is not clear. Shafer (83) stated that the lazy and normal plants used were "nearly always from seeds from the same ear; but since this seed was not inbred the plants must have varied much in spite of care used to select similar ones." Nonetheless Shafer interpreted these results to suggest that lazy corn is positively geotropic. Shafer also observed that lazy plants become aphototropic at about the same time as they become ageotropic.

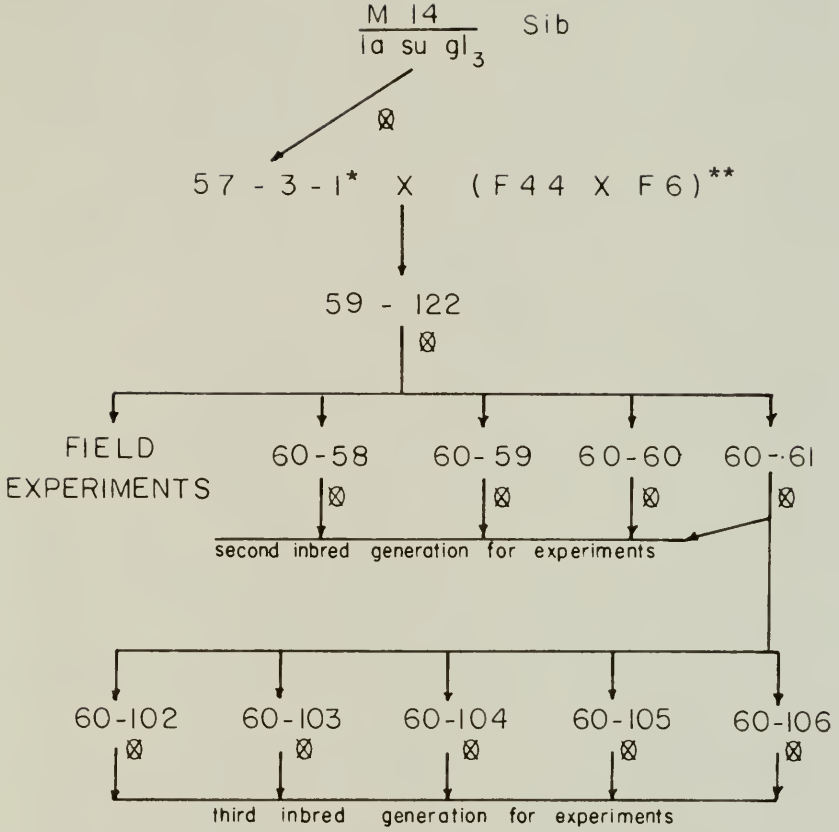
CHARACTERISTICS OF THE PLANT MATERIAL

Materials and Methods

Plant production

The corn plants used in this study were derived from Maize Genetics Cooperative stock 50-409-1/-2 which had the genotype (Ml4/la su gl_3) sibling. A crossing program (Figure 3) was initiated to produce inbred lines adapted to Florida growing conditions which would segregate for the lazy character. A secondary objective of the program was to obtain these lines free of the sugary (su) character which is linked with lazy (39). First generation inbred seeds were used for field experiments; second and third generation inbred seeds were used as a source of plants for laboratory experiments.

Plants for laboratory experiments were raised in the greenhouse under a minimum photoperiod of twelve hours. Night temperatures were maintained at 65° F; day temperatures never exceeded 95° F. The plants were raised in six-inch clay pots filled with fumigated soil. Four seeds from a single line were planted per pot. During early stages of growth the plants in each pot were irrigated twice weekly



*Year-line-plant number.

**(F44 x F6) is one of the parents of Dixie 18, a hybrid widely grown in the South.

Fig. 3.--Crossing plan and utilization of inbred lines segregating for the lazy character.

with about 150 cc of Hoagland's solution. The plants were irrigated with this nutrient solution daily, after the onset of rapid growth just prior to tasseling. No visible deficiency symptoms were observed.

Immediately after the onset of rapid growth the geotropic reactivity of the plants was determined by placing the pot and plants horizontally. Those plants which exhibited a bending response within 48 hours were tentatively classified as normal and those which did not respond classified as lazy. At this time two of the four plants in the pot were removed. Whenever possible, a normal plant and a lazy plant were left in the pot. Presumptive lazy plants were tied to bamboo stakes placed vertically in the pots. Reorientation of the pot vertically gave an additional check on the geotropic reactivity of the presumed normal plants. Plants were classified as normal if they responded to gravity after these two reorientations and if they subsequently maintained a vertical orientation. Growth of a lazy plant above the point at which it was tied to the bamboo stake was accompanied by a curvature of this new growth away from the vertical. A presumptive lazy plant was classified as lazy if it had to be tied frequently to the bamboo stake to maintain it in a vertical orientation. The latter

criterion was also used for classification of lazy plants in field plantings.

Plants were taken for laboratory experiments after they had tasseled. The plants were kept in the dark at 25±1° C overnight in the laboratory before each experiment to minimize both the effect of seasonal differences in the greenhouse environment and any disturbing effect incurred in transporting the plants.

Breaking strength and growth of stems

Jenkins and Gerhardt (36) proposed that the lazy habit of growth resulted from the structural weakness of the stem. Before examining the geotropic behavior of the lazy mutant stock on hand it was necessary to test this hypothesis. A field experiment to examine breaking strength and growth of normal and lazy siblings employed a randomized block design with six replications. Each block was four rows wide with ten plants per row. A row was planted with seed from one of the four selected first generation selfed plants. The expected segregation in these lines is one lazy plant to three normal plants. As a consequence of this segregation, the experimental results were evaluated by covariance analysis to adjust for unequal numbers of the two plant types (85).

The height of the plants was estimated immediately after pollen was shed and stem elongation had essentially stopped. Height was measured from the ground level to the first node below the tassel. The breaking strength of the stems was estimated immediately thereafter by a modification of the method employed by Rogers (72). One end of a piece of strong cord was looped around the center of the second internode above the ground. The other end of the cord was attached to a 100 pound capacity spring balance. The breaking strength was taken to be the equivalent of the force in pounds required to break the stem when the balance was pulled slowly and steadily in a horizontal direction.

Geotropic reaction of lazy and normal corn

One hundred of the first generation selfed seed were sown in two-inch wooden plant bands filled with soil and then grown for two weeks before being transplanted to the field. The geotropic reaction of these seedlings was determined while they were in the coleoptile stage by placing the wooden bands on their sides. The bands remained in this orientation for two days during which time the coleoptiles of the plants ruptured, and the first two leaves expanded. The bands were then reoriented vertically and the geotropic reaction of the seedlings again measured. The seedlings

were then planted in the field where they were subsequently classified as lazy or normal.

Results

Breaking strength and growth of stems

Measurements of the breaking strength of stems of normal and lazy corn segregating after one generation of inbreeding are given in Table 2. The mean breaking strength of the 60 lazy plants was 33.1 pounds, of the 151 normal plants 34.6 pounds. The adjusted means were 31.4 pounds for lazy plants and 34.5 pounds for normal plants. Covariance analysis indicated that there was no significant difference between the breaking strengths of normal and lazy plant stems.

Growth measurements of these normal and lazy plants are given in Table 3. The average height of the 60 lazy plants was 43.9 inches, of the 151 normal plants 47.3 inches. The adjusted means were 44.1 inches for lazy and 48.5 inches for normal. There was no significant difference in height between normal and lazy plants. There was, however, a significant difference in the heights of plants between these first generation inbred lines. The adjusted mean heights per plant for these lines were 46.2, 51.4, 45.9, and 41.8 inches.

Table 2.--BREAKING STRENGTH OF FIRST GENERATION INBRED CORN SEGREGATING FOR THE LAZY CHARACTER

Block	Plant Type	Plants	Total Breaking Strength	Plants	Total Breaking Strength	Plants	Total Breaking Strength	Plants	Total Breaking Strength
		no.	lbs.	no.	lbs.	no.	lbs.	no.	lbs.
1	Lazy	0	0	2	75	1	44	2	64
	Normal	8	205	6	226	8	271	8	247
2	Lazy	4	132	3	112	2	40	5	109
	Normal	4	110	6	196	4	111	4	117
3	Lazy	1	37	4	143	2	64	1	25
	Normal	9	355	6	207	8	254	8	284
4	Lazy	5	231	3	104	2	33	3	99
	Normal	5	284	7	244	7	218	7	281
5	Lazy	2	62	4	99	2	63	2	86
	Normal	6	187	6	160	7	215	5	221
6	Lazy	3	81	2	111	4	134	1	37
	Normal	6	230	5	194	5	181	6	217

Table 2--Continued

Analysis of Covariance

Source of Variation	d.f.	Sums* x^2	Sums* xy	Sums* y^2	Deviations from Regression f	Deviations from Regression Sum $d_{y.x^2}$	Deviations from Regression Mean Square
Blocks (B)	5	6.354	352.875	25,728.000			
Line (L)	3	0.229	21.333	3,982.500			
Type (T)	1	172.521	6,123.542	217,352.083			
LxT	3	2.896	121.292	6,203.417			
Error							
LxB	15	9.396	59.292	4,113.400	14	3,759.247	268.518
TxB	5	37.354	1,156.083	38,298.917	4	2,518.869	629.717
LxTxB	15	30.729	1,053.583	55,071.683	14	18,948.245	1,353.446
Total Error (E)	35	77.479	2,268.958	97,484.000	34	31,037.990	912.882
L+E	38	77.708	2,290.291	101,466.500	37	33,963.375	
For testing adjusted mean differences for lines					3	2,925.385	975.128
T+E	36	250.000	8,392.500	314,836.083	35	33,099.858	
For testing adjusted mean difference for type					1	2,061.868	2,061.868
LxT+E	38	80.375	2,390.250	103,687.417	37	32,604.430	
For testing adjusted mean differences for LxT					3	1,566.440	522.147

*x is number of plants; y is breaking strength.
Effects not significant at the 0.05 level.

Table 3.--HEIGHT OF FIRST GENERATION INBRED CORN SEGREGATING FOR THE LAZY CHARACTER

Block	Plant Type	Line 1 Plants	Line 1 Total Height	Line 2 Plants	Line 2 Total Height	Line 3 Plants	Line 3 Total Height	Line 4 Plants	Line 4 Total Height
		no.	in.	no.	in.	no.	in.	no.	in.
1	Lazy	0	0	2	101.5	1	50.5	2	58.5
	Normal	8	282.5	6	327.0	8	352.5	8	245.0
2	Lazy	4	156.5	3	144.0	2	90.5	5	191.5
	Normal	4	161.0	6	272.0	4	161.0	4	153.0
3	Lazy	1	51.0	4	179.0	2	103.0	1	55.0
	Normal	9	491.5	6	359.5	8	414.0	8	387.5
4	Lazy	5	207.0	3	115.0	2	92.5	3	109.0
	Normal	5	249.0	7	326.0	7	308.5	7	320.5
5	Lazy	2	68.5	4	176.5	2	98.0	2	100.5
	Normal	6	279.0	6	259.5	7	315.0	5	246.0
6	Lazy	3	152.0	2	103.5	4	183.0	1	45.5
	Normal	6	328.5	5	296.5	5	283.0	6	322.0

Table 3--Continued

Source of Variation	d.f.	Analysis of Covariance: Sums*** x^2	Sums*** xy	Sums*** y^2	Deviations from Regression: f	Deviations from Regression: Sum $d_{y.x^2}$	Deviations from Regression: Mean Square
Blocks (B)	5	6.354	346.229	41,010.917			
Line (L)	3	0.229	34.792	7,592.875			
Type (T)	1	172.521	8,546.417	423,376.334			
LxT	3	2.896	65.458	1,690.458			
Error (E)							
LxT (1)	15	9.396	289.646	18,671.125	14	9,742.346	695.882
TxB (2)	5	37.354	1,658.146	85,244.287	4	11,639.100	2,909.775**
LxTxB (3)	15	30.729	1,193.729	52,380.171	14	6,007.399	429.100
Error (1)+(3)	30	40.125	1,483.375	71,051.296	29	16,212.632	559.056
L+E (1)+(3)	33	40.354	1,518.167	78,644.171	32	21,528.865	
For testing adjusted mean differences for lines					3	5,316.233	1,772.078*
T+E (2)	6	209.875	10,204.563	508,620.621	5	12,453.350	
For testing adjusted mean difference for type					1	814.250	814.250
LxT+E (1+3)	33	43.021	1,548.833	72,741.754	32	16,980.994	
For testing adjusted mean differences for LxT					3	768.362	256.121

**Indicates significant at the 0.01 level; *0.05 level. The other effects are not significant at the 0.05 level.

***x is number of plants; y is height.

Geotropic reaction of lazy and normal corn

In the coleoptile stage the seedlings which were proven by subsequent testing to be lazy had bent an average of 88.3 degrees four hours after reorientation; normal plants had bent an average of 87.6 degrees in the same period. After the coleoptile had ruptured, lazy plants bent an average of only 3.4 degrees in 48 hours after reorientation, whereas normal plants bent an average of 40.3 degrees. Five days after reorientation normal plants were vertical but lazy plants still averaged only about three degrees of bend.

The geotropic reactivity of normal corn plants was also examined in more mature plants. Five days after horizontal placement, 50 six-week-old potted plants exhibited an average curvature of only 39.7 degrees resulting from bending at only one node. Young plants continued to respond in this manner up to the period of rapid elongation which commences approximately 60 days after planting. At this time plants become more responsive to gravity and remain so for most of their subsequent life. Average bending of 90 degrees is usually achieved in about 48 hours with the bending distributed over four or five nodes (Figure 2). As the plants mature, the nodes which respond are located further

up the stem, presumably since lower nodes have lost their potential for elongation. For example, when silks appear on the ear, the ear node has largely lost its potential to respond to gravity.

Growth habit of lazy corn plants

In the course of growing corn for the crossing program pits were dug next to several lazy plants after they had started to bend over. As anticipated, the lazy corn stems continued to bend until the stems were vertical with the tassel pointing downward (Figure 4). This inverted growth habit is taken to be the typical habit of growth of mature lazy plants.

Discussion

Both Van Overbeek (89) and Shafer (83) observed that young lazy corn seedlings responded to gravity. These observations were confirmed in the present study with the qualification that only coleoptiles of lazy seedlings are capable of responding to gravity. Young seedling stems are not responsive. The expression of the lazy gene is apparently manifested in the stem but not in the coleoptile of lazy plants. The expression of this gene, in the genetic stock examined, was not associated with significant effects

Fig. 4.--The uninhibited growth habit of lazy corn plants. The pit into which this plant is growing was dug after the plant had started to bend.

on either the growth or the breaking strength of stems of lazy plants.

The coleoptiles of normal corn seedlings respond to gravity at a much faster rate than young seedling stems. As the stem elongates, however, it becomes progressively more responsive to gravity. The response is maximal and involves four or five nodes subsequent to the period of rapid elongation of the stem. The experiments reported in subsequent sections utilized plants in this growth period when there was a maximum amount of geotropically responsive tissue.

GEOTROPIC ASSAY OF ISOLATED CORN STEM SEGMENTS

Methods

A convenient assay was needed to test the effect of various compounds on the geotropic reaction of corn stems. The assay was particularly needed to test the effect of IAA since it was planned to use IAA in transport experiments.

Corn stems were defoliated one internode at a time, and the stem cut into segments one cm below each node. The morphological base of the stem segment was placed immediately in an appropriate solution one cm deep. The upper end of the segment was inserted into tight-fitting tubing attached to an aspirator and a vacuum applied for one minute. In preliminary tests with safranin, the dye appeared at the upper end of the segment 20 to 30 seconds after the application of vacuum. Subsequent dissection of the stem showed red dye associated with the vascular strands.

After infiltration, the section was trimmed two cm below the node and five cm above, and the distal end of the segment covered with parafilm. The segment was then oriented horizontally by inserting its base into a block of water-saturated plastic foam (Oasis brand). The angle of

the segment from the horizontal was determined. If the angle deviated more than five degrees, the segment was re-inserted. The block of plastic foam with the stem segments was placed in a chamber in the dark at a temperature of $25 \pm 1^{\circ}$ C and a relative humidity of 90 to 95 percent. The angle of bend of a segment from the horizontal was determined after 96 hours.

The chemicals employed were all dissolved in 0.02 M pH 6.0 potassium phosphate buffer. The buffering capacity of the phosphate was adequate for all compounds used. IAA was first dissolved in an equimolar amount of 0.10 N sodium hydroxide (5 mg IAA equivalent to 0.286 ml of base) with a magnetic stirrer before being diluted with phosphate. Larsen (48) stated that a five-fold excess of base is required to affect solution of IAA. One lot of IAA (Nutritional Biochemicals Corporation), however, dissolved in less than 10 minutes; another lot of IAA (Fisher Lot 794428) did not dissolve in two hours. These lots of IAA have identical melting point ranges which agree with literature values and equivalent biological activity in the standard Avena test, and in reversing radiation inhibition of the geotropic reaction.[1] Both lots produce only one spot upon chromatography

[1] H. J. Teas and T. W. Holmsen, unpublished experiments.

with two solvents and three color reagents. Apparently these lots of IAA differ only in their physical form. The Nutritional Biochemicals Corporation product is supplied in the form of platelets, whereas the Fisher product is granular. Because of the difference in solubility, only the Nutritional Biochemicals Corporation IAA was used.

Results

In preliminary tests with the assay, the eight apical nodal sections of 87-day-old second inbred generation plants were all treated with the same compound (Table 4). The type of response observed is shown in Figure 5. (The plant material for these photographs came from a later experiment.) NP and 2,5-dinitrophenol (DNP) inhibited geotropic reactivity of normal corn, as reported by Larsen (51) and others for a number of plants. The apparent stimulation by deionized water and 10^{-4} M NaCN might have been an artifact. In further preliminary screening, this apparent stimulation was not observed, nor did 10^{-4} M 2,3,5-triiodobenzoic acid and 10^{-4} M chlorogenic acid have an effect. However, 10^{-3} M 2,3,5-triiodobenzoic acid markedly inhibited the geotropic reaction as in the case of other plants (71, 87).

The average angle of bend for the reacting nodes exhibited a maximum at the fourth node from the apex

Table 4.--THE EFFECT OF VARIOUS REAGENTS ON THE ANGLE OF BEND OF NODAL STEM SECTIONS OF SECOND INBRED GENERATION CORN

Normal Plants

Progeny	Treatment**	Node Number*								
		1	2	3	4	5	6	7	8	Sum
		Degrees								
61-11	Buffer	0	10	20	17	8	5	0	0	60
58-8	Deionized water	12	21	29	30	30	0	0	0	122
61-11	10^{-5} M IAA	5	8	14	2	10	0	0	0	39
61-11	10^{-4} M IAA	3	5	13	13	7	8	12	2	63
60-2	10^{-5} M NP	0	5	5	12	22	18	15	0	77
60-6	10^{-4} M NP	0	0	0	0	0	0	0	0	0
60-6	10^{-4} M NaCN	0	32	34	36	18	7	6	7	140
61-11	10^{-3} M NaCN	0	16	11	19	0	0	0	0	46
60-6	10^{-4} M DNP	0	3	12	20	17	0	0	0	52
59-1	10^{-3} M DNP	0	0	0	0	0	0	0	0	0
Avg.-Nodes of Reacting Plants		2	12	17	19	14	5	4	1	

Lazy Plants

Progeny	Treatment**	Node Number*								
		1	2	3	4	5	6	7	8	Sum
		Degrees								
61-11	Buffer	0	0	0	6	10	0	0	0	16
58-8	Deionized water	0	0	0	0	0	8	0	0	8
61-11	10^{-5} M IAA	0	0	0	0	0	0	0	0	0
61-11	10^{-4} M IAA	0	6	0	0	7	4	7	0	24
60-8	10^{-5} M NP	0	0	0	0	0	0	0	0	0
60-6	10^{-4} M NP	9	5	31	11	12	9	0	0	77
60-6	10^{-4} M NaCN	0	0	0	0	0	0	0	0	0
61-2	10^{-3} M NaCN	0	0	0	0	0	0	0	0	0
61-16	10^{-4} M DNP	0	0	0	0	0	0	0	0	0
58-5	10^{-3} M DNP	0	0	0	0	0	0	0	0	0

*Node number 1 is the apical node.

**The concentration listed for a compound refers to the concentration infiltrated and not to the concentration in the tissue.

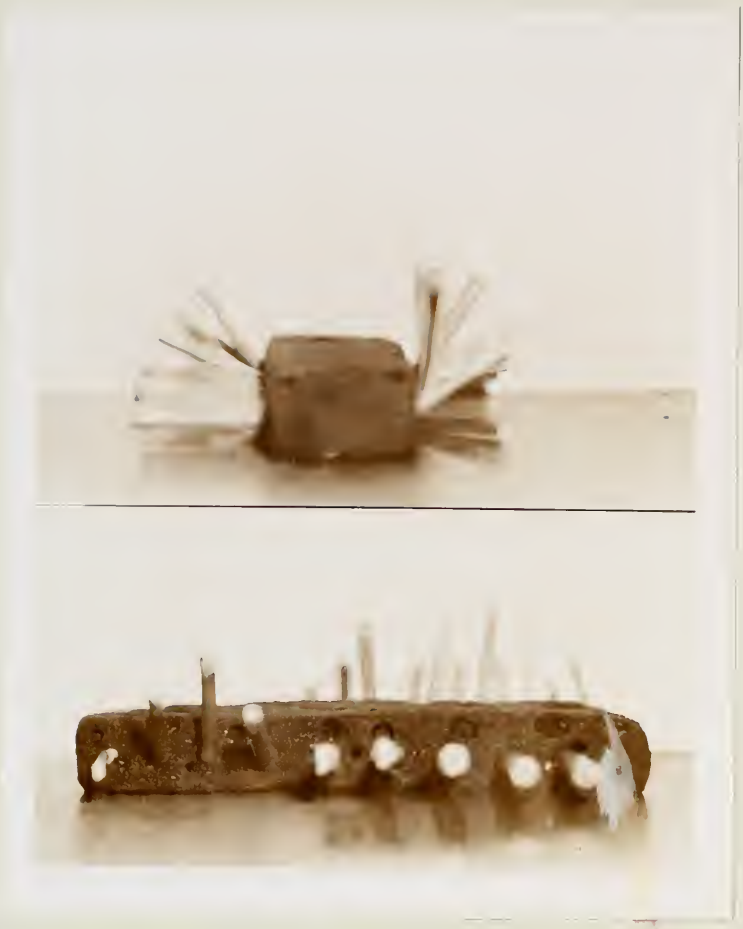


Fig. 5.--Corn node segments in plastic foam 96 hours after horizontal placement of the segments. The segments on the left in the upper view correspond to the segments in front in the lower view. In the lower view, the fifth segment from the right is the ear node. Note that segments proximal to the ear node are relatively unreactive, whereas distal nodes exhibit a marked response to gravi y.

(Table 4). The reactivity of nodes distal to the fourth node fell off gradually; that of proximal nodes was more abrupt. This abrupt reduction occurred at the ear node (see Figure 5).

It is significant to note that nodal sections from lazy plants did not exhibit a positive geotropic reaction (Table 4). In fact, after infiltration with either buffer or deionized water an occasional node exhibited a negative geotropic reaction. The results suggest that NP may cause nodal sections of lazy plants to become negatively geotropic. This compound was selected for more intensive study.

The results of an experiment employing five levels of NP are presented in Table 5. A Latin square design (85) was employed with a pot containing both sibling lazy and normal plants as columns and nodal sections as rows. The corresponding nodes of normal and lazy plants growing in the same pot received the same treatment. This design permitted a comparison of the reactions of lazy and normal plants to the compound. The experimental plants were 87-day-old third generation siblings. NP clearly reduced the geotropic reaction of normal nodal segments. However, there was no significant trend in the effect of NP with respect to concentration. As in the screening experiments (Table 4), nodal

Table 5.--THE EFFECT OF NP ON THE ANGLE OF BEND PER NODE OF STEM SEGMENTS FROM SIBLING NORMAL AND LAZY CORN PLANTS

Treatment	Normal Plants	Lazy Plants
	degrees	degrees
Buffer	29.0	7.5
10^{-5} M NP	6.8	4.3
$5x10^{-5}$ M NP	2.3	6.5
10^{-4} M NP	14.8	-0.5
$5x10^{-4}$ M NP	9.5	6.3
10^{-3} M NP	4.3	2.5

Analyses of Variance

I

Source of Variation	d.f.	Mean Square
Sums		
Pots	5	112.368
Nodes	5	77.258
Treatment		
Buffer vs. NP	1	1,575.025**
Remainder	4	41.807
Error (a)	20	111.247
Differences		
Normal vs. lazy (C)	1	806.681*
Pots x C	5	154.780
Nodes x C	5	144.547
Treatment x C		
Buffer vs. NP	1	789.136*
Remainder	4	151.817
Error (b)	20	181.240

Table 5--Continued

II

Source of Variation	d.f.	Mean Square
Normal Corn		
Pots	5	143.312
Nodes	5	179.712
Treatment		
Buffer vs. NP	1	2,296.939**
Remainder	4	142.322
Error (a)	20	269.836
Lazy Corn		
Pots	5	123.778
Nodes	5	42.044
Treatment		
Quartic	1	193.393**
Remainder	4	19.707
Error (b)	20	22.678
Comparison (+ vs. la)	1	806.681

**Significant at the 0.01 level; *significant at the 0.05 level.

segments from lazy plants exhibited a negative geotropic reaction although significantly less than that of normal plants. On the average, NP treatment did not differ significantly from buffer treatment of lazy plants. There was a significant quartic trend in the effect of NP, however, on the geotropic reaction of nodal stem segments of lazy plants (Figure 6).

The results of this experiment were confirmed in a similar subsequent experiment; that is, the geotropic reaction of nodal stem segments from normal plants was reduced by NP but the effect did not exhibit significant maxima, minima, or trend in the concentration range 10^{-5} M to 10^{-3} M NP. Nodal stem segments from lazy plants exhibited a negative geotropic reaction which was significantly less than that of normal segments. The quartic trend of reactivity of nodal stem segments from lazy plants with concentration of NP was again observed (Figure 6).

In three experiments with a total of 108 nodal stem segments from lazy plants, no effect of IAA in the concentration range of 10^{-5} to 10^{-3} M was observed. This result was expected since Van Overbeek (90) found that lazy plants contain a slightly greater auxin content than normal plants.

The effect of IAA on geotropic bending of nodal stem

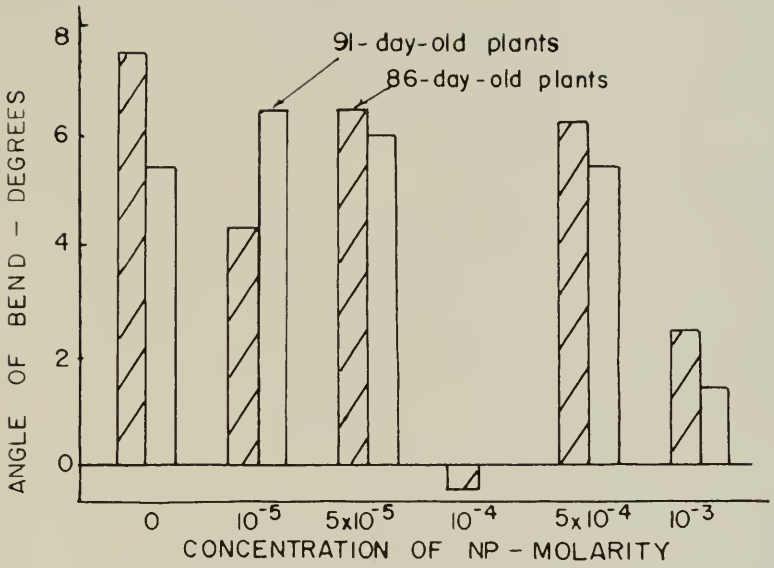


Fig. 6.--Response of nodal stem segments of lazy corn to various concentrations of NP. Each bar represents the average of six observations.

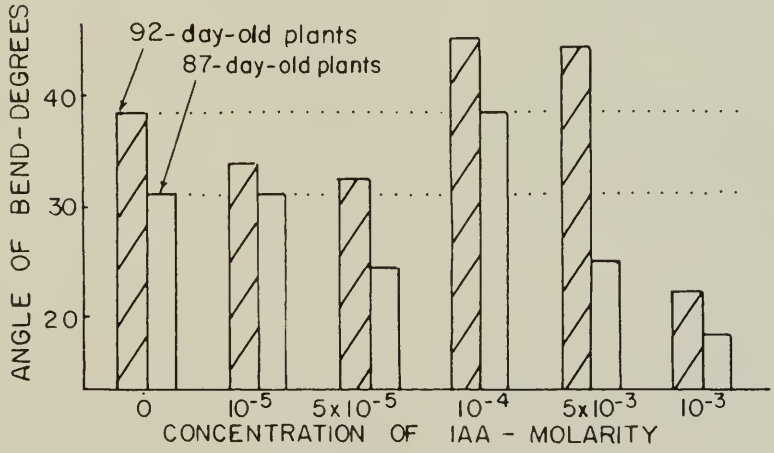


Fig. 7.--Response of nodal stem segments of normal corn to various concentrations of IAA. Each bar represents the average of six observations. Dotted line represents level of response of control.

segments from normal plants was examined in a Latin square design (85) with plants as columns and nodal segments as rows. Bending was increased when nodal stem segments were infiltrated with 10^{-4} M IAA (Figure 7). However, a significant effect of IAA treatment on the geotropic response of these segments could not be demonstrated (Table 6). Concentrations of 10^{-4} M and 5×10^{-4} M IAA also stimulated bending in a subsequent experiment (Figure 7). A significant effect of IAA treatment, however, could not be demonstrated.

Discussion

The fact that isolated nodal segments of corn stems respond to gravity indicates that these segments contain the entire geotropic apparatus. Reduction in geotropic reactivity resulting from NP, DNP, and 2,3,5-triiodobenzoic acid, therefore, can not be attributed to any one phase of the geotropic reaction on the basis of this assay.

IAA is known to exist in large quantities (105,000 microgram IAA equivalents per kg fresh weight) in endosperm of corn seed (53). Housley and co-workers (35), however, could find no chromatographically identifiable IAA in extracts of four-day-old corn seedlings. Apparently there is no record of attempts to identify the auxin(s) of mature corn plants.

Table 6.--THE EFFECT OF IAA ON THE ANGLE OF BENDING PER NODE OF STEM SEGMENTS FROM NORMAL CORN PLANTS

Treatment	Degrees
Buffer	32
10^{-5} M IAA	31
$5x10^{-5}$ M IAA	25
10^{-4} M IAA	38
$5x10^{-4}$ M IAA	26
10^{-3} M IAA	19

Analysis of Variance

Source of Variation	d.f.	Mean Square
Plants	5	1,973.600
Nodes	5	1,922.733
Treatment	5	180.467
Error	20	390.000

Treatment effect not significant at the 0.05 level.

The effect of IAA concentration on geotropic bending of corn node segments (Figure 7) follows a typical IAA response curve in going through an optimum (53). It is not surprising that a significant effect of IAA was not observed since horizontal placement of corn stems results in an increased production of auxin (83). Brandes and McGuire (8) observed an effect of IAA on the geotropic response of sugar cane stems only after treating the stems for 20 minutes at 52^{o} C. A similar depletion of auxin from corn nodal segments should permit detection of a significant effect of IAA on geotropic bending of the segments.

Nodal stem segments from lazy corn plants exhibit a slight, but real, negative geotropic reaction. The existence of this reactivity is confirmed by the observation that NP inhibits geotropic bending of the segments. The facts at hand do not permit identification of the deficiency in the geotropic reaction inherent in lazy corn. It is, however, capable of perception of the stimulus of gravity, at least to a limited degree. It is also significant that nodal stem segments from lazy plants do not exhibit a positive geotropic reaction.

TRANSPORT OF IAA

Materials and Methods

As a logical extension of the Cholodny-Went theory one might expect to observe an increase in lateral transport of exogenous auxin from the upper to the lower surface of a horizontally placed stem as compared to transport from the lower surface to the upper surface. IAA transport in corn stems was examined in isolated blocks of internal tissue (ground parenchyma and vascular tissue [20]) taken from growth rings of corn stems. The blocks were cut from the morphological center of the stem by means of two parallel single edge ejector-type razor blades mounted with their long axis perpendicular to the jaws of a jewelers vise. The size of the blocks varied from experiment to experiment.

Two lots of IAA 2-C^{14} were used in these experiments. The first lot (Nuclear-Chicago) had a specific activity of 21.7 microcuries per mg, and the second (Orlando Research) 6.58 microcuries per mg. Both lots were examined by ascending filter paper strip chromatography in two solvents (water-saturated n-hexane and butanol:acetic acid:water,

10:1:1,v:v:v) and with three indole color reagents: p-dimethylaminobenzaldehyde (53), p-dimethylaminocinnamaldehyde (28), and $FeCl_3$-$HClO_4$ (53). In all cases IAA was the only compound discovered on the chromatograms. After color development the filter paper strips were cut perpendicularly to the direction of solvent flow into one cm segments and the radioactivity in the segments determined by counting them directly. Determinations of radioactivity were made with a gas flow counter with a "micromil" window and operating in the Geiger region. In all cases the peak of radioactivity coincided with the color spot on the chromatogram.

The methods outlined by Larsen (48) were adopted for making solutions of IAA and IAA agar. The radioactive IAA was dissolved in redistilled ether and stored until used in cork-stoppered brown glass bottles at 5° C. A given amount of the ether solution was evaporated in a test tube in a water bath at 50° C by passing $CaCl_2$ dried air at 1.5 p.s.i. into the tube. As soon as the ether was evaporated, equal quantities of 0.02 M pH 6 citrate buffer and 0.004 M $CaCl_2$ were added to the tube which was then stoppered and set aside at room temperature for 30 minutes. Filtered-autoclaved 2.5 percent agar which had been melted and then cooled to 50° C was then added to the tube. The final

concentrations of the various components were 1.4×10^{-4} M IAA, 0.01 M pH 6 citrate buffer, 0.002 M $CaCl_2$, and 1.25 percent agar. The warm agar was pipetted into 2.0 mm thick stainless steel forms to solidify. The agar blocks were trimmed to the thickness of the form and then cut into platelets with the same cross-sectional area as the tissue block to which they were subsequently applied. Recipient agar platelets were made in the same manner with the omission of radioactive IAA.

The agar platelets were applied to freshly cut tissue blocks; a platelet containing IAA 2-C^{14} being applied to one side of the block and recipient agar containing no IAA placed on the opposite side. The various combinations of tissue configuration and orientation are shown in Figure 8. The tissue and agar platelets on shallow aluminum planchets were placed in a humid chamber for the period of transport. Transport was carried out in diffuse light (2-3 foot candles) at a temperature of 25 ± 1^{o} C and a relative humidity of 90-95 percent. At the termination of the time for transport the tissue block was removed and the agar dried directly on the planchet under infrared light. The radioactivity of the dried agar platelet was then determined.

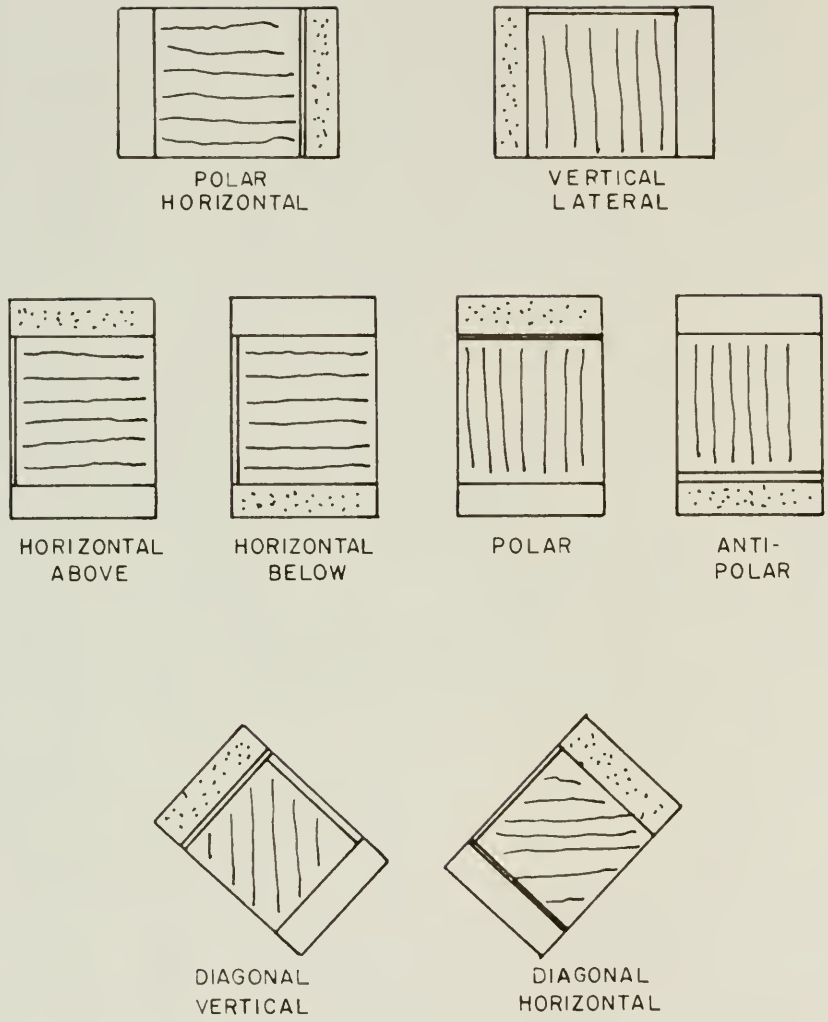


Fig. 8.--Configurations of blocks of corn stem tissue and agar to study transport of IAA through the tissue. Lines on the tissue indicate orientation of vascular tissue. Heavy double line indicates morphologically upper end of block. Stippled area represents donor agar containing 2-C^{14} IAA.

Results

Losses of radioactivity from tissue blocks

It was necessary to learn whether IAA 2-C^{14} was metabolized to CO_2 by corn tissue blocks and whether tissue orientation had an effect. Tissue blocks were infiltrated with IAA 2-C^{14} in a vertical orientation from donor agar platelets at both the proximal and distal ends of the block. Three hours after placement of the agar the tissue blocks were removed from the agar and bisected vertically. One-fourth of the bisected blocks were put immediately into formalin-acetic acid-alcohol (FAA); one-fourth remained vertical; one-fourth were placed horizontally so that both halves became upper halves; and one-fourth placed horizontally so that both halves became lower halves. After 24 hours in the dark at 25 ± 1^{o} C and 92-95 percent relative humidity the last three groups of blocks were also placed in FAA. The radioactivity removed from these blocks by three extractions with FAA is tabulated in Table 7. There was no evidence for catabolism of the added IAA 2-C^{14} to CO_2 during the 24-hour period following administration of the isotope. In transport experiments, then, no correction need be made for losses of radioactivity during the experimental period.

Table 7.--THE EFFECT OF TIME ON THE AMOUNT OF RADIOACTIVITY REMAINING IN TISSUE BLOCKS FROM CORN STEMS AFTER INFILTRATION WITH IAA 2-C^{14}

Conditions of the Experiment

Plants: Third inbred generation normal (102-11 and 102-7); 87 days old; shedding pollen
Internodes: 4-7 from apex
Tissue blocks: 4 x 4 x 4 mm
IAA: 5×10^{-4} M; about 10,100 cpm per donor platelet
Design: Two 4 x 4 Latin squares with plants as squares, internodes as columns, and position on internode as rows

Treatment	Radioactivity per Block
	cmp
Radioactivity determined immediately	7,546
Radioactivity determined 24 hours after:	
horizontal placement-lower halves	7,443
horizontal placement-upper halves	7,770
vertical placement	7,913

Analysis of Variance

Source of Variation	d.f.	Mean Square
Plants	1	90,979,933
Internodes within plants	6	2,192,672
Position in internode within plants	6	1,629,151
Treatments	3	361,864
Remainder	3	1,210,573
Combined error	12	970,623

Treatment effect not significant at the 0.05 level.

Characteristics of lateral transport

The time-course of lateral transport was examined for both the horizontal-above and horizontal-below configurations. Diagrams of the appropriate configurations are shown in Figure 8 and Tables 8 and following. In this experiment, a randomized block design (85) with plants as replications, tissue blocks were cut only from nodes of the stem. The same tissue blocks were used throughout the eight-hour transport period, but the recipient agar platelet was replaced one, two, and four hours after commencement of the experiment. In the eight hour transport period, there was no significant difference in the amount transported between the two orientations (Table 8).

The time-course for transport is shown in Figure 9. After an initial lag period of about 1.14 hours, lateral transport in horizontally placed stems was linear with time. If the concentration gradient in the tissue block is linear, an apparent diffusion coefficient, D, for lateral transport may be calculated (Table 9). The table includes estimates from a subsequent experiment with the horizontal-above configuration ("Horizontal-above-2").

The diffusion coefficient, D^{25}, estimated from the results of these experiments ranged from 1.18×10^{-7} to

Table 8.--LATERAL TRANSPORT OF RADIOACTIVE IAA THROUGH HORIZONTALLY PLACED CORN STEM BLOCKS DURING AN EIGHT-HOUR PERIOD

Conditions of the Experiment

Plants: Second inbred generation normal (60-8, 60-5); 61 days old; pre-tassel stage
Nodes: 8 and 9 from apex
Tissue blocks: 7.5 x 7.5 x 4 mm; transport through 4 mm direction
Time: 8 hours
IAA: 1.15×10^{-4} M, about 38,900 cpm per donor platelet

Orientation	Average Radioactivity in Recipient Agar
	cpm
	63.3
	55.0

Analysis of Variance

Source of Variation	d.f.	Mean Square
Plants	9	846.44
Orientation	1	346.11
Error	9	120.82

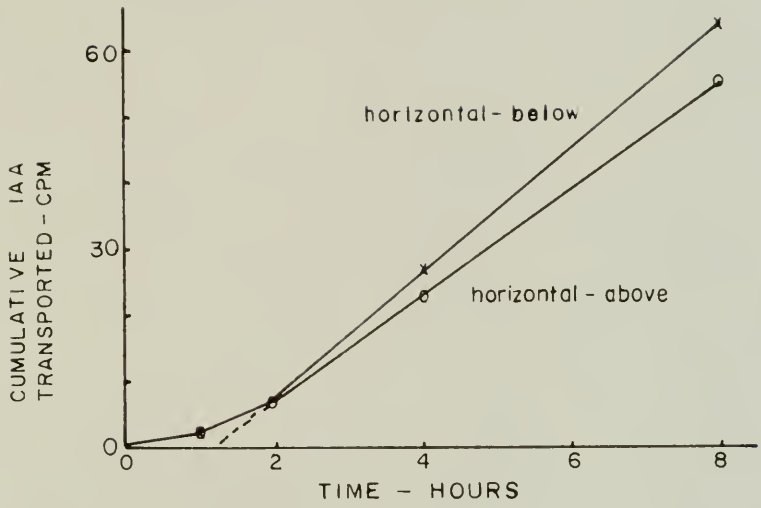


Fig. 9.--Time-course of lateral transport of radioactive IAA in horizontally placed corn stem tissue blocks.

Table 9.--CALCULATION OF THE DIFFUSION CONSTANT, D, OF IAA BASED ON LATERAL TRANSPORT THROUGH CORN STEM TISSUE BLOCKS

Parameter	Corn Tissue Blocks		
	Horizontal-Below	Horizontal-Above-1	Horizontal-Above-2
(1) Activity in donor block-cpm	38,950	38,950	10,846
(2) Volume of donor block-cc	0.113	0.113	.0321
(3) IAA concentration moles/liter	1.15×10^{-4}	1.15×10^{-4}	5.0×10^{-4}
(4) Rate of transport cpm/hr	9.25	7.93	13.1
(5) $\frac{dQ}{dt} = \frac{(2)(3)(4)}{3600(1)}$ moles/sec	8.77×10^{-15}	7.46×10^{-15}	5.41×10^{-14}
(6) a-cm^2	0.562	0.562	0.16
(7) Δ x-cm	0.4	0.4	0.4
(8) D^{25}-cm^2/sec	5.41×10^{-7}	4.60×10^{-7}	1.18×10^{-7}

$$\frac{dQ}{dt} = -Da \frac{dC}{dx}$$

5.41×10^{-7} cm^2 per sec. These values for lateral transport of IAA through corn stem sections are considerably lower than the value of 7.45×10^{-6} cm^2 per sec for the diffusion of IAA through 1.5 percent agar calculated by Larsen (48).

In one of the subsequent transport experiments (Table 14) the average distribution of radioactivity at the end of a six-hour transport period was 3,735 cpm in the donor agar platelet, 6,380 cpm in the tissue, and 35 cpm in the recipient agar platelet. The amount of radioactivity in the tissue was determined by difference from the total amount added, 10,150 cpm (average of 10 determinations). IAA diffusion was carried out in a stack of two mm agar platelets concurrently with the transport experiment. At the end of three hours, the distribution of radioactivity in these platelets was 3,953 cpm in the donor agar platelet, 3,042 cpm in the next lower agar platelet, 1,862 cmp in the next lower agar platelet, and 1,389 in the lowest agar platelet. Radioactivity in a parallel stack of platelets reached equilibrium at the end of six hours. It should be noted that the stack of agar platelets was one mm thicker than the stack of agar and tissue block combined.

If IAA were moving through the tissue block by a process of diffusion and were not accumulated by the tissue,

then a linear concentration gradient would exist in the tissue block. Under these conditions, the tissue block would contain (3,700)(1.5/2) or about 2,670 cpm. Clearly, then, IAA accumulates in the tissue.

The effect of DNP on lateral transport of IAA was examined in an experiment employing two nodal sections from each of four normal plants. The concentration of DNP used, 10^{-3} M, was found to inhibit the geotropic response of corn nodal sections (Table 4). Nodal sections were infiltrated with either DNP or buffer (0.02 M potassium phosphate, pH 6.0) and after one hour 5 x 5 x 3 mm tissue blocks were cut from the nodes of the sections. Transport was carried out in the horizontal-above configuration for three hours with 5 x 10^{-5} M IAA 2-C^{14} in the donor agar. Radioactivity in the recipient agar platelets of buffer-treated tissue blocks was 11.2$\pm$1.5 cpm. The radioactivity of recipient agar platelets of DNP-treated tissue blocks was 11.2$\pm$2.4 cmp. DNP at a concentration which inhibits the geotropic reaction of nodal stem segments did not inhibit the lateral transport of IAA 2-C^{14} through tissue blocks from these segments.

Since there is a gradient of geotropic responsiveness along the successive nodes of a corn stem (Table 4, Figure

5), lateral transport was examined with respect to nodal position on the stem. The results of an experiment measuring transport in the horizontal-above configuration (Table 10) failed to indicate any such gradient. The slope of the regression line (0.0176) of lateral transport compared to node position did not differ significantly from zero (t=0.0157, 32 d.f.). Although there was considerable variation between plants, there was no significant deviation from parallelism between the regression lines for the individual plants (85).

Effect of gravity on lateral transport

As shown in Table 8, transport in the horizontal-below configuration was slightly greater than transport in the horizontal-above configuration, but the difference was not significant. Transport in these configurations was examined in several additional experiments. In the first of these, an evaluation was made of the effect of the orientation of the stem prior to the transport measurements. One group of plants was placed horizontally 12 hours before tissue blocks were taken from them; a comparable group of plants remained vertical during this time. As shown in Table 11, there was no significant difference in transport between the horizontal-above and horizontal-below configurations regardless of the orientation of the stem prior to measurement of

Table 10.--LATERAL TRANSPORT OF RADIOACTIVE IAA IN BLOCKS OF CORN STEM TISSUE IN THE HORIZONTAL-ABOVE CONFIGURATION AS A FUNCTION OF NODAL POSITION

Conditions of the Experiment

Plants: Second inbred generation normal (59-1, 59-18, 60-8, and 61-11); 63 days old; pre-tassel stage
Nodes: 6-10 from apex
Tissue blocks: 7.5 x 7.5 x 4 mm; transport through 4 mm direction
Time: 2 hours
IAA: 1.15×10^{-4} M, about 38,900 cpm per donor platelet

Node	Transport of IAA
	cpm
6	13.9
7	15.4
8	13.6
9	14.7
10	16.9

Analysis of Variance

Source of Variation	d.f.	Mean Square
Plants	7	308.26
Joint Regression	1	1.26
Parallelism	7	.37
Error	18	28.54

Table 11.--EFFECT OF TISSUE ORIENTATION BEFORE TRANSPORT ON LATERAL TRANSPORT OF RADIOACTIVE IAA

Conditions of the Experiment

Plants: Second inbred generation normal (60-6 and 60-10); 56 days old; pre-tassel stage
Nodes: 9-11 from apex
Tissue blocks: 7.5 x 7.5 x 4 mm; transport through 4 mm direction
Time: 1 hour
IAA: 1.15×10^{-4} M, about 38,900 cpm per donor platelet

Orientation	Average Radioactivity in Recipient Agar	
	Stems Vertical before Experiment	Stems Horizontal 12 Hours before Experiment
	cpm	cpm
	4.91	4.49
	6.11	4.08

Analyses of Variance

Source of Variation	d.f.	Stems Vertical before Experiment	Stems Horizontal 12 Hours before Experiment
		Mean Square	Mean Square
Plants	7	17.09	4.73
Orientation	1	5.76	0.68
Error	7	12.64	11.44

transport. This and a subsequent experiment (Tables 11, 12) originally included a comparison of the horizontal transport configurations with the vertical-lateral orientation (Figure 8). An artifact resulted, hence the data are not included in Tables 11 and 12.

A comparison was made of lateral transport in lazy as well as normal plants. The experimental design employed was a randomized block (85) with a pot containing a normal and lazy plant taken as a block. The analysis of the experiment followed procedures outlined previously (Table 5) modified to a randomized block design.

The results of the experiment (Table 12) again indicated no significant difference in lateral transport in the horizontal-above and horizontal-below configurations. No effect of gravity on lateral transport could be demonstrated in either normal or lazy tissue blocks. Lateral transport in tissue blocks from lazy stems was less than lateral transport in comparable tissue from normal stems but the difference fell short of significance ($F=5.23$; $F_{.05}=6.61$). It should be noted that the normal and lazy plants compared were not siblings but were from sister lines which differ by one generation of independent segregation.

A method which permitted evaluation of lateral

Table 12.--THE EFFECT OF GRAVITY ON LATERAL TRANSPORT OF RADIOACTIVE IAA IN TISSUE BLOCKS FROM STEMS OF NORMAL AND LAZY CORN

Conditions of the Experiment

Plants: Second inbred generation normal (61-16) and lazy (61-2) growing in the same pot; 75 days old; shedding pollen
Nodes: 7-9 from apex
Tissue block: 7.5 x 7.5 x 3 mm; transport through 3 mm direction
Time: 2 hours
IAA: 1.15×10^{-4} M, about 38,900 cpm per donor platelet

Configuration	Average Radioactivity in Recipient Agar	
	Normal Plants	Lazy Plants
	cpm	cpm
	17.2	7.9
	16.6	13.9

Analysis of Variance

Source of Variation	d.f.	Mean Square
Sums		
Pots	5	45.14
Orientation	1	63.69
Error (a)	5	81.49
Differences		
Lazy vs. Normal (C)	1	217.80
Pots x C	5	80.51
Orientation x C	1	45.10
Error (b)	5	41.61

transport in the vertical-lateral orientation consisted of placing the tissue block on the edge of a glass microscope slide held firmly in a horizontal staining dish. The placement of the tissue block was such that neither donor nor recipient agar platelet came in contact with the glass slide, thus preventing the artifact obtained in previous trials.

An experiment to compare the three configurations of lateral transport with polar transport (Figure 8) again failed to demonstrate any influence of gravity on lateral transport in either normal or lazy plants (Table 13). The results indicated that polar transport was about three times as fast as lateral transport. This difference was significant. As in the previous experiment, tissue blocks from lazy plants transported less than tissue blocks from normal plants. This difference was significant in the present experiment, which had a higher sensitivity than the previous one. It should be noted again that this comparison of normal and lazy plants was made between sister lines and not between sibling plants.

In all of the foregoing transport experiments, tissue blocks were taken from median sections of nodal tissue, that is, all sections included the morphological center of the stem. Measurements of lateral transport in such horizontally

Table 13.--LATERAL TRANSPORT OF RADIOACTIVE IAA IN TISSUE BLOCKS OF NORAML AND LAZY CORN STEMS AS AFFECTED BY GRAVITY AND AS COMPARED TO POLAR TRANSPORT

Conditions of the Experiment

Plants: Second generation inbred normal (61-16) and lazy (61-2) growing in the same pot; 77 days old; shedding pollen
Nodes: 6-9 from apex
Tissue blocks: 7.5 x 7.5 x 3 mm; transport through 3 mm direction
Time: 3 hours
IAA: 1.15×10^{-4} M, about 38,900 cpm per donor platelet

Orientation	Average Radioactivity in Recipient Agar	
	Normal Plants	Lazy Plants
	cpm	cpm
	14.3	5.0
	9.2	6.5
	9.3	5.2
	32.0	17.1

Analysis of Variance

Source of Variation	d.f.	Mean Square
Sums		
Pots	7	34.56
Transport		
Polar vs. Lateral	1	3,176.88**
Remainder	2	24.41
Error (a)	21	114.98
Differences		
Lazy vs. Normal (C)	1	956.36**
Pots x C	7	110.43
Transport x C	3	124.11
Error (b)	21	89.51

**Indicates significance at the 0.01 level.

placed tissue blocks estimate the net effect of transport in the upper and lower halves of the block. If the effect of gravity were to induce an increase in lateral transport in one half of a horizontally placed stem and a proportionate decrease in lateral transport in the other half, then the methods employed would not detect this change.

Nodal tissue was cut longitudinally into thirds and the center third discarded in an experiment to measure lateral transport separately in upper and lower halves of the stems. Tissue blocks cut from the peripheral thirds were made "upper halves" by placing the edge formed by the original longitudinal cut downward. Similarly, tissue blocks were made "lower halves" by placing the edge formed by the original longitudinal cut upward. Two nodes were used per plant. The two tissue blocks from one node were made either "upper" or "lower." One of the blocks was used to measure lateral transport of IAA from the lower surface of the block to the upper surface. The other block was used to measure lateral transport from the upper surface to the lower surface of the block. A split-plot design was employed (85). No differences were observed in either the overall capacity to transport or the capacity to transport laterally in either direction (Table 14).

Table 14.--LATERAL TRANSPORT OF IAA 2-C^{14} IN HORIZONTAL TISSUE BLOCKS FROM THE PERIPHERY OF CORN STEM NODES

Conditions of the Experiment

Plants: Third generation inbred normal (102-2, 102-11, and 106-4); 84 days old; shedding pollen
Nodes: 7 and 8 from apex
Tissue blocks: 4 x 4 x 3 mm; transport in 3 mm direction
Time: 6 hours
IAA: 5×10^{-4} M, about 10,100 cpm per donor platelet

Orientation	Direction of Transport	Average Radioactivity in Recipient Agar
		cpm
Upper	From Above	34.3
Upper	From Below	39.7
Lower	From Above	29.3
Lower	From Below	35.1

Analysis of Variance

Source of Variation	d.f.	Mean Square
Main plots		
Plants	4	283.60
Orientation: Upper vs. Lower (O)	1	114.24
Error (a)	4	1,412.79
Sub-plots		
Transport: Above vs. Below (T)	1	155.68
O x T	1	270.05
Error (b)	8	443.89

Effect of gravity on polar transport

In all lateral transport experiments, the radioactive IAA was supplied to the tissue block chiefly through the parenchyma of the stem and perhaps occasionally through one or two vascular bundles exposed in cutting the block. If auxin is normally supplied to the stem through vascular tissue, then failure to demonstrate the influence of gravity on lateral transport may result from the mode of application of the radioactive IAA. The diagonal configurations (Figure 8) were adopted to examine this possibility. In these configurations, the tissue block is supplied with IAA from both the polar and lateral directions. They provide a measure of net lateral and polar transport in one half of a tissue block as limited by lateral transport from the other half of the block.

Experiments on lateral transport utilized tissue blocks only from the growth rings (nodes) of the stems since geotropic bending is manifested in this area. This difference in response between the growth ring and the remainder of the internode may result from the difference in growth potential and not from intrinsic differences in the capability to transport auxin. In preliminary experiments to work out techniques for examining transport in the diagonal

configurations, the average transport for eight tissue blocks cut from the growth ring was 18.1 cpm and was 20.4 cpm for similar tissue blocks cut from the adjacent internodal segments.

As a result of these observations, the experimental design previously employed was modified. A pot containing a normal and lazy plant was taken as the basic unit for a 4 x 4 Latin square with internodes as columns and position on the internode as rows. Tissue blocks and agar in the diagonal configuration were placed on planchets held at an angle of 45 degrees to the horizontal. The tissue blocks in the polar-horizontal configuration (Figure 8) rested on the edge of a glass microscope slide in a manner similar to that described for the vertical-lateral orientation. As in all previous experiments, there was no evidence to support the hypothesis that gravity influences lateral transport of exogenously applied IAA (Table 15). The experimental results did show, however, that polar transport was significantly greater than transport in the polar-horizontal configuration, that is, gravity reduced polar transport in horizontally placed tissue blocks. Furthermore, these results confirmed the observation made in the previous experiment that polar transport of IAA was significantly greater than

Table 15.--TRANSPORT OF IAA IN THE POLAR AND LATERAL DIRECTIONS IN BLOCKS OF NORMAL AND LAZY CORN STEM TISSUE AS AFFECTED BY GRAVITY

Conditions of the Experiment

Plants: Second inbred generation segregating normal and lazy (58-12); 106 days old
Internodes: 2-5 from apex
Tissue blocks: 3 x 3 x 3 mm
Time: 3 hours
IAA: 10^{-4} M, about 6,200 cpm per donor platelet

Orientation	Average Radioactivity in Recipient Agar	
	Normal Plants	Lazy Plants
	cpm	cpm
	44.9	35.3
	23.1	29.3
	17.9	25.3
	20.0	22.3

Table 15.--Continued

Analysis of Variance

Source of Variation	d.f.	Mean Square
Sums		
Plants	2	1,589.66
Internodes in plants	9	356.58
Position on internode in plants	9	595.68
Orientation		
Polar vs. Diagonal	1	3,324.08**
Polar vs. Polar-Horizontal	1	2,329.65*
Diagonal vs. Diagonal-Horizontal	1	2.39
Residual	6	455.39
Error (a)	18	378.57
Differences		
Normal vs. Lazy (C)	1	56.89
Plants x C	2	2,138.06
Internodes in plants x C	9	320.15
Position on internode in plant x C	9	133.05
Orientation x C	3	361.79
Residual	6	304.70
Error (b)	18	337.51

**Indicates significance at the 0.01 level; *0.05 level.

lateral transport. There was also no significant difference in IAA transport between sibling lazy and normal plants.

The influence of gravity on polar transport was examined in another experiment with hybrid normal plants (Table 16). Gravity again reduced the polar transport of IAA in horizontally placed tissue blocks. The influence of gravity in these two experiments (Tables 15 and 16) was relatively the same, causing a reduction in polar transport of about 45 to 48 percent.

Characteristics of polar transport

NP and DNP both inhibit the geotropic reaction of corn stems (Tables 4 and 5) and are known to reduce polar transport in other species (58, 60). Therefore, the effect of these compounds on polar transport of IAA in corn stem tissue blocks was examined. Nodal stem segments were infiltrated with the reagents as in the geotropic assay of isolated corn node segments. Each stem segment was divided laterally into three sections and two tissue blocks cut from each section. One tissue block was placed in the polar orientation, the other block in the polar-horizontal orientation. A split-plot design was employed for the experiment (85).

Table 16.--INFLUENCE OF GRAVITY ON POLAR TRANSPORT OF IAA IN BLOCKS OF NORMAL CORN STEM TISSUE

Conditions of the Experiment

Plants: Hybrid; 89 days old; had shed pollen
Nodes: 4 and 5 from apex
Tissue blocks: 7.5 x 7.5 x 3 mm; transport through 3 mm direction
Time: 3 hours
IAA: 10^{-4} M, about 6,200 cpm per donor platelet

Orientation	Average Radioactivity in Recipient Agar
	cpm
	40.8
	22.3

Analysis of Variance

Source of Variation	d.f.	Mean Square
Internodes	5	224.04
Orientation	1	851.93**
Error	5	47.05

**Indicates significance at the 0.01 level.

The extent of participation of xylem and adjacent lacunae in polar transport was also evaluated. If air is pulled through a stem section, transport through these elements is prevented. In the present experiment, nodal stem segments were infiltrated with buffer (0.02 M potassium phosphate, pH 6.0), air was pulled through one-half of these infiltrated segments, and then tissue blocks cut from them.

NP and DNP greatly reduced polar transport in corn stems (Table 17) as in other species of plants (58, 60). The action of these compounds was such that no further reduction of transport was observed in horizontally placed tissue blocks. Transport observed in these tissue blocks might be attributed to diffusion of IAA through the xylem and lacunae. To test such a suggestion, however, one must know how long it takes for these reagents to reach a locus of action so that subsequent air treatment will not remove them.

The experimental results also showed that about 40 percent of polar transport as previously measured is attributable to transport through xylem and lacunae. The portion of the IAA thus transported was not influenced by gravity. There was, however, a significant influence of gravity on that portion of the IAA transported by non-lacunar tissue.

Table 17.--THE INFLUENCE OF GRAVITY ON POLAR TRANSPORT OF IAA IN CORN STEM TISSUE BLOCKS INFILTRATED WITH DNP, NP, BUFFER, OR AIR

Conditions of the Experiment

Plants: Second inbred generation normal (58-8); 84 days old; shedding pollen
Internodes: 3-6 from apex
Tissue blocks: 3 x 3 x 3 mm
Time: 3 hours
IAA: 1 x 10^{-4} M; about 34,800 cpm per donor platelet

Treatment	Average Radioactivity in Recipient Agar	
		
	cpm	cpm
10^{-3} M DNP	5.9	5.8
10^{-4} M NP	6.3	6.0
Air	11.9	3.9
Buffer	20.0	11.7

Analysis of Variance

Source of Variation	d.f.	Mean Square
Main plot		
Plants	1	0.91
NP vs. DNP (A)	1	0.58
Buffer vs. air (B)	1	381.60**
Buffer+air vs. NP+DNP (C)	1	418.90**
Error (a)	19	3.48
Sub-plot		
Polar vs. Polar-Horizontal (O)	1	210.84**
A x O	1	0.20
B x O	1	0.48
C x O	1	190.32**
Error (b)	20	10.46

**Indicates significance at the 0.01 level.

This component of polar transport was reduced about 60 percent by gravity. Transport in the polar and anti-polar (Figure 8) configurations was measured as part of another experiment. Four tissue blocks were used for each configuration for both normal and lazy plants. The tissue blocks were 7.5 x 7.5 x 3 mm, and transport was carried out for two hours. Anti-polar (acropetal) transport occurred in corn stem tissue, but to a lesser extent than polar transport (Table 18). Lazy and normal plants apparently have equivalent capacities for these two kinds of transport. The results of the previous experiment (Table 17) suggest that a considerable portion of transport in the acropetal direction may have resulted from diffusion through lacunae in the tissue.

Table 18.--COMPARISON OF POLAR AND ANTI-POLAR TRANSPORT IN TISSUE BLOCKS FROM NORMAL AND LAZY CORN STEM NODES

Plant Type	Average Radioactivity in Recipient Agar 	
	cpm	cpm
Normal	26.4±12.8*	14.6±1.2
Lazy	25.1±2.8	17.8±11.3

* ± standard error.

Polar transport in tissue blocks from sibling normal and lazy corn was again compared. Under comparable experimental conditions with 24 nodal tissue blocks from each plant type, polar transport in normal tissue blocks resulted in 9.1 ± 2.8 cpm in the recipient agar, and polar transport in lazy corn resulted in 9.4 ± 4.7 cpm. Thus, there are three independent observations (Tables 15 and 18) that polar transport in lazy plants does not differ from polar transport in normal plants.

Discussion

The rate of lateral transport of IAA in corn stems is only about 5 percent of the rate of diffusion of IAA through agar (Table 10). During transport the tissue blocks were observed to contain more radioactive IAA than could be accounted for by a linear concentration gradient (Page 71). As shown in calculations in the Appendix, cells with a uniform capacity on all surfaces to pump a particular compound into them are incapable of accumulating that compound. Since parenchyma cells appear to be physiologically uniform in all directions, they would be unable to accumulate IAA even if they possessed the capacity to pump it into the cells. The high concentration of IAA in the cells and the limited rate of lateral transport through them may,

therefore, result from binding but not accumulation of the IAA in the cells.

DNP decreases both polar transport (Table 18) and geotropic bending (Table 5) but has no effect on lateral transport. These facts suggest that lateral transport is a permeation process rather than an active transport process.

The results of six independent experiments (Tables 9 and 12 to 15) involving 50 pairs of tissue blocks failed to demonstrate any effect of gravity on lateral transport. Furthermore, the gradient of geotropic reactivity with nodal position (Table 5) is not associated with a gradient in capacity for lateral transport (Table 11). About 60 percent of the diffusible auxin of corn stems is obtained from the lower half of the stem. If lateral transport were responsible for this unequal distribution then the ratio of lateral transport from the lower to the upper half of the stem to that from the upper to the lower half would be about 0.67. The weighted mean ratio observed in these experiments was 0.989. These experiments were more sensitive to changes in lateral transport than previous investigations (12, 15, 70) since the IAA flux instead of accumulation in the tissue was measured.

These data suggest, therefore, that processes other than lateral transport are responsible for the unequal

distribution of diffusible auxin in horizontally placed stems.

Polar transport of IAA, in contrast to lateral transport, is reduced about 60 percent by gravity (Table 18). Since gravity does not affect transport through xylem and lacunae (Table 18) it is inferred that gravity affects polar transport in phloem or in parenchyma. Influence on the latter is less probable, although by no means rejected, since the parenchyma cell appears to be morphologically and physiologically the same in all directions. Furthermore, lateral transport must occur through parenchyma, and there is no effect of gravity on lateral transport.

NP and DNP both reduce polar transport (Table 18) such that no gravity effect is observed. These observations coupled with those of previous investigations (61, 95) suggest that polar transport is an active metabolic process influenced by gravity. The results thus lead to the tentative hypothesis that phloem is a site of geotropic perception and that this perception results in a reduction in polar transport of IAA through the phloem. The hypothesis accounts for the necessary cellular requirements for geotropic perception. The geotropic reaction necessitates that cells not only sense the direction of the force of gravity but also

their relationship to the morphological center of the reacting organ. The location, morphology, and origin of phloem would appear to confer on it the necessary attributes to fulfill these two requirements.

Examination of IAA transport in lazy plants did not produce any new information regarding transport. Comparisons between normal and lazy siblings revealed no significant differences in transport between the two types of plants. Significant differences were observed, however, between lazy and normal plants from sister lines. Since these lines differ by one generation of independent segregation, the differences observed may have resulted from gene action trivial to the lazy phenotype.

DISPOSITION OF IAA IN TISSUE

Methods

As an alternative to the Cholodny-Went theory, the form of auxin in tissue may be altered under the influence of gravity. Studies on the uptake of auxin by tissue have indicated that auxin may exist in cells in at least three forms which have been provisionally classified as diffusible auxin, exchangeable auxin, and bound auxin (37, 69). Experiments were designed to study the influence of gravity on the distribution of IAA among these forms in tissue blocks from corn stems.

IAA 2-C^{14} was introduced into tissue blocks by the same methods employed in transport experiments. The tissue blocks were then blotted with absorbent paper and cut in half. One-half was placed in two ml of deionized water in a deep planchet, the other in two ml of IAA solution. The orientation of the tissue block was maintained while it was in the solution by impaling it on the end of a fine Siliclad-coated wire looped over the edge of the planchet. The time-course of elution of radioactivity from the tissue block was followed by transferring the tissue block

periodically with blotting to a fresh solution. Except for experiments in which temperature was a variable, elution was carried out at 25±1° C. Radioactivity in the solutions was then determined after drying them under infrared light.

After elution the tissue blocks were fixed in FAA for 24 hours, dehydrated in a standard t-butyl alcohol series, and inbedded in Tissuemat. Imbedded tissue was sectioned at 20 microns and fixed to round coverslips. The sum of the radioactivities of the FAA, dehydrating alcohol, and thin sections represented the radioactivity remaining in the tissue after elution in the solutions. It was subsequently discovered that all of the radioactivity remaining in the tissue could be removed by three successive treatments with FAA of 24, 4, and 4 hours duration, respectively. This method was employed in later experiments.

Results

In a preliminary experiment, IAA 2-C^{14} was introduced into 3 x 3 x 3 mm tissue blocks in the polar and polar-horizontal orientations. The tissue blocks were cut into halves parallel with the orientation of the vascular bundles. One of the halves was placed into deionized water and the other half into 10^{-4} M IAA. No attempt was made to keep the tissue blocks oriented in the solutions. The blocks were

transferred to fresh solutions 0.5, 1.0, 1.5, and 2.0 hours after being placed into the initial solution.

The time-course of release of radioactivity into these solutions is shown in Figure 10. The initial half-hour elution period is omitted from Figure 10 since the tissue blocks were not blotted before being placed in the solution.

It can be seen from Figure 10 that more radioactivity was eluted from the tissue blocks into IAA solution than into water. It is also apparent that three hours elution in either water or IAA solution was insufficient to deplete the elutable radioactivity in the tissue block. The results suggested, furthermore, that the form of IAA in the tissue might be affected by tissue orientation.

Effect of gravity and NP

The differences in the form of IAA in tissue blocks in the polar and polar-horizontal orientations were examined more fully in a split-plot design (85). Nodal stem segments of sibling lazy and normal plants were infiltrated with either buffer or NP. The stem segments were divided transversely into three parts and two tissue blocks cut from each part. IAA 2-C^{14} was introduced into one of the tissue blocks in the polar orientation and into the other in the polar-horizontal orientation. After three hours of

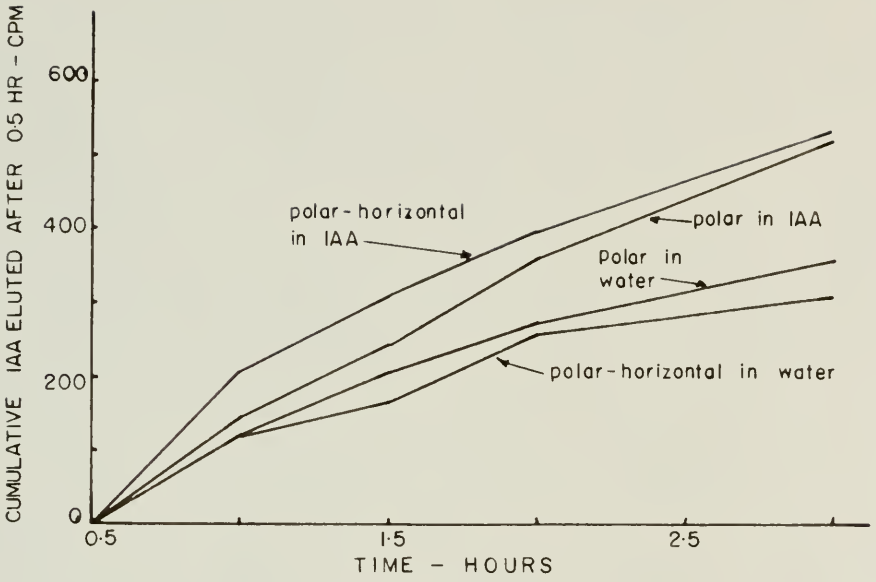


Fig. 10.--Time-course of release of radioactivity from tissue blocks of corn stem nodes into water or 10^{-4} M IAA.

transport the agar platelets were removed and the tissue blocks bisected. One-half of the block was placed in deionized water, the other half in 10^{-4} M IAA. The original orientation of the block was maintained while it was in the solution. The blocks were transferred with blotting to fresh solutions 10, 40, 100, and 220 minutes after starting elution and were placed in FAA after 460 minutes. The elution period was extended from three hours to nearly eight hours to allow for more complete elution.

The total amount of radioactivity recovered from tissue blocks from both normal and lazy plants was unaffected by orientation (Table 19). NP significantly increased the amount of radioactive IAA contained in tissue blocks from lazy plant stems but had no effect on the total radioactivity in normal tissue.

The elution of radioactive IAA from tissue blocks, as shown in Figures 11 to 14, was rapid at first. At the end of 460 minutes, the rate of release was much slower than at the end of the elution period in the previous experiment (Figure 10). In experiments to be discussed later (Figures 15 and 16), radioactive IAA was still being released into water after 24 hours. This suggests that the amount of IAA irreversibly bound in tissues is negligible, and that all of

Table 19.--TOTAL RADIOACTIVITY IN TISSUE BLOCKS FROM NORMAL AND LAZY CORN STEMS AFTER THREE HOURS TRANSPORT

Conditions of the Experiment for Tables 19-21

Plants: Second inbred generation normal and lazy (58-8); 84 days old; shedding pollen
Internodes: 3-6 from apex
Tissue blocks: 3 x 3 x 3 mm
Time: 3 hours transport; 460 minutes elution
IAA: 1×10^{-4} M, about 34,800 cpm per donor platelet

Chemical	Orientation	Total Activity per Tissue Block	
		Normal	Lazy
		cpm	cpm
Buffer		5194	4450
		5322	4725
NP		5028	5943
		5305	5124

Analyses of Variance

Source of Variation	d.f.	Mean Square	
		Normal	Lazy
Main plot			
Plants	1	20,296	210,278
Chemical (C)	1	12,449	3,941,667**
Error (a)	9	925,579	155,026
Sub-plot			
Orientation (O)	1	92,314	15,444
O x C	1	6,697	356,903
Error (b)	10	650,657	216,991

**Indicates significance at 0.01 level.

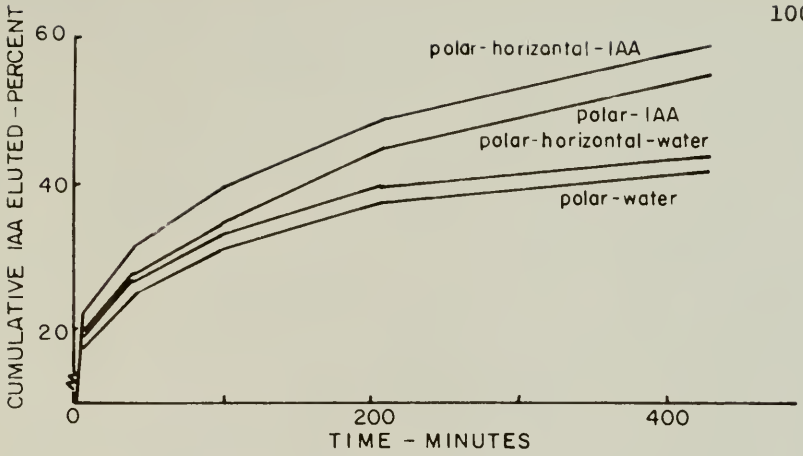


Fig. 11.--Time-course of elution of radioactive IAA from tissue blocks of normal corn stems infiltrated with buffer.

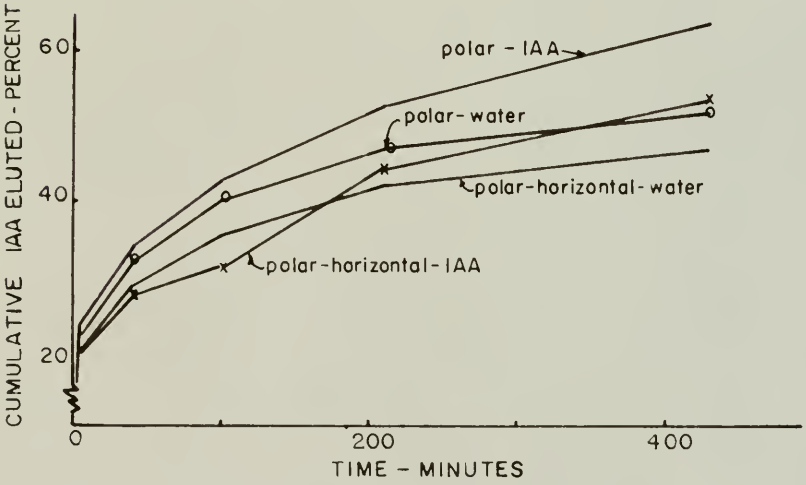


Fig. 12.--Time-course of elution of radioactive IAA from tissue blocks of normal corn stems infiltrated with NP.

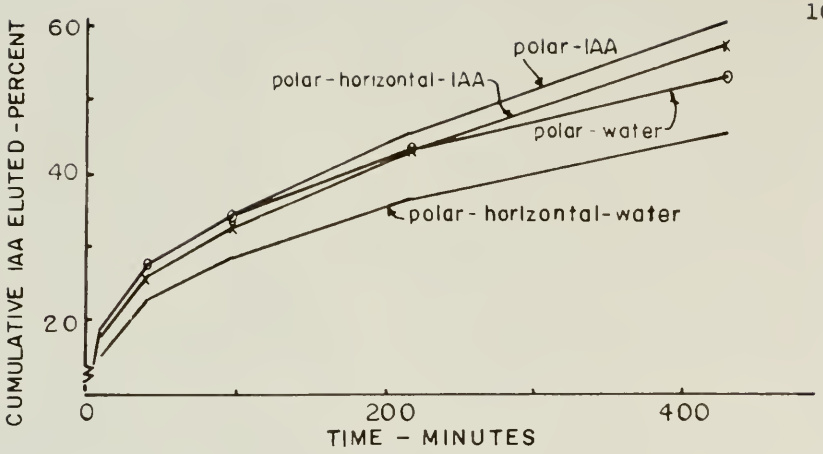


Fig. 13.--Time-course of elution of radioactive IAA from tissue blocks of lazy corn stems infiltrated with buffer.

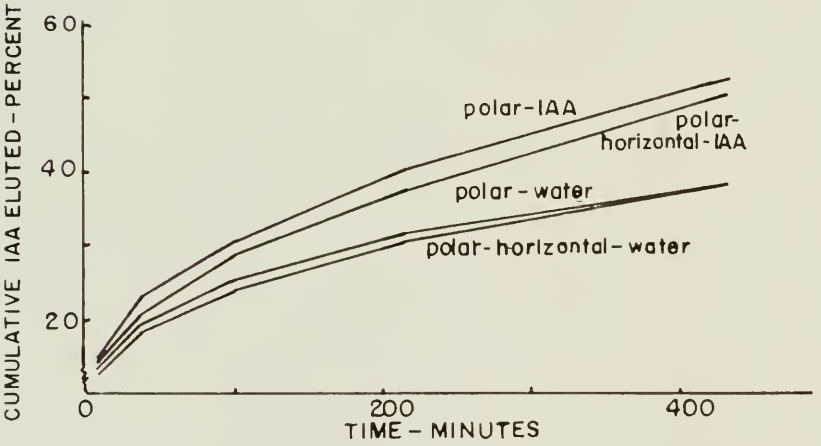


Fig. 14.--Time-course of elution of radioactive IAA from tissue blocks of lazy corn stems infiltrated with NP.

the IAA should eventually be eluted. This reasoning led to plotting 100 minus the percent eluted versus time on semi-logarithmic paper. The resulting curve was observed to have two components and was fitted by the expression

$$E = 100 - Re^{-k_r t} - Se^{-k_s t}$$

where E is the percent radioactive IAA eluted in time, t, from a pool comprising R percent of the total radioactivity with a rapid rate of elution, k_r, and from a pool comprising S percent of the total radioactivity with a slow rate of elution, k_s. R may represent freely diffusible radioactive IAA and S exchangeable and/or actively accumulated IAA. This equation predicts that, given sufficient time, all of the radioactivity would be eluted from the tissue blocks.

The parameters of the equation estimated from semi-logarithmic plots of the elution data shown in Figures 11 to 14 are given in Table 20. The radioactive IAA in the rapidly eluted component, R, comprised about 20 to 40 percent of the total radioactivity of the tissue and was eluted with a half-time of about 20 to 40 minutes. The radioactivity in the slowly eluted component, S, was eluted with a half-time in the range of 10 to 30 hours.

Horizontal placement of tissue blocks from buffer-infiltrated normal corn stems was associated with a transfer

Table 20.--PARAMETERS OF $E = 100 - Re^{-k_r t} - Se^{-k_s t}$ OBTAINED FROM THE DATA SHOWN IN FIGURES 11 TO 14

Plant Type	Chemical	Orientation	Parameter			
			R	k_r	S	k_s
			percent	per hour	percent	per hour
		Elution with water				
Normal	Buffer	Polar	22.0	0.920	78.0	0.0376
		Polar-horizontal	33.5	.900	66.5	.0215
	NP	Polar	42.3	1.08	57.7	.0261
		Polar-horizontal	37.8	.986	62.2	.0215
Lazy	Buffer	Polar	32.0	.811	68.0	.0498
		Polar-horizontal	28.0	.964	72.0	.0360
	NP	Polar	25.5	1.07	74.5	.0243
		Polar-horizontal	23.5	1.14	76.5	.0284
		Elution with IAA				
Normal	Buffer	Polar	31.5	0.797	68.5	0.0538
		Polar-horizontal	37.8	.932	62.2	.0503
	NP	Polar	43.0	1.17	57.0	.0627
		Polar-horizontal	36.0	1.16	64.0	.0428
Lazy	Buffer	Polar	31.5	1.42	68.5	.0715
		Polar-horizontal	24.0	2.15	76.0	.0753
	NP	Polar	29.5	1.03	70.5	.0519
		Polar-horizontal	25.7	1.15	74.3	.0498

of radioactive IAA from pool S to pool R, a 43 percent reduction in k_s, but no marked changes in k_r. The presence of NP in polar-oriented normal tissue was associated with these same kind of changes. Upon horizontal placement of NP-treated normal tissue, however, radioactive IAA was transferred from pool R to pool S and the reduction in k_s was only slight.

Horizontal placement of tissue blocks from buffer-infiltrated lazy corn stems was associated with a decrease in k_s as in the case of NP-treated normal tissue. Horizontal placement of NP-treated lazy tissue blocks produced no marked change in any parameter of the system.

The effect of IAA as an eluant was associated with an increase in k_s regardless of treatment. Tissue blocks from buffer-treated normal stems exhibited a shift of radioactive IAA from S to R in response to IAA as an eluant. NP inhibited this transfer in normal tissue blocks. This transfer was also absent in tissue blocks from buffer-infiltrated lazy corn stems but was observed to a slight extent in tissue blocks from NP-infiltrated lazy stems. The presence of IAA in the eluting solution did not markedly effect k_r in normal stem tissue blocks. In contrast, k_r in tissue blocks from buffer-infiltrated lazy stems increased about one and

a half times in the presence of IAA in the eluting solution. This increase was not observed in NP-infiltrated lazy tissue blocks.

An analysis of variance of the parameters of the equations was not made. The elution data (Figures 11 to 14) satisfy the assumption of normality, so the percentage of radioactivity obtained from the tissue in 100 minutes was analyzed. In this period of time, 81 to 97 percent of the radioactivity in the R pool was released as compared to only 4 to 11 percent of the radioactivity in the S pool.

There was a significant interaction between tissue orientation and NP treatment on the amount of radioactive IAA eluted from normal stem tissue into both water and IAA (Table 21). Horizontal placement of buffer-infiltrated normal stem tissue blocks was associated with an increase in the amount of radioactive IAA obtained into both IAA and water in 100 minutes. In contrast, horizontal placement of NP-infiltrated tissue blocks was associated with a decrease in the amount of radioactive IAA so obtained.

The amount of radioactive IAA eluted from tissue blocks from lazy corn stems was not affected by orientation (Table 21). NP treatment, however, was associated with a significant decrease in the amount of IAA eluted into water.

Table 21.--THE EFFECT OF GRAVITY ON THE FORM OF RADIOACTIVE IAA IN TISSUE BLOCKS FROM NORMAL AND LAZY CORN STEMS INFILTRATED WITH NP AND BUFFER

Orientation	Chemical	100 minute "Diffusible" IAA***		100 minute "Exchangeable" IAA***	
		Normal	Lazy	Normal	Lazy
		percent	percent	percent	percent
	Buffer	30.7	34.6	4.1	0.1
	NP	40.6	25.4	2.6	5.3
	Buffer	33.0	28.9	6.4	3.8
	NP	35.7	23.9	-5.1	4.2

Analyses of Variance

Source of Variation	d.f.	Mean Square "Diffusible" IAA		Mean Square "Exchangeable" IAA	
		Normal	Lazy	Normal	Lazy
Main plots					
Plants	1	50.17	116.16	261.38	325.51
Chemical (C)	1	237.51	299.63**	78.50	46.24
Error (a)	9	110.35	43.26	68.74	53.45
Sub-plots					
Orientation (O)	1	10.53	80.67	47.06	5.88
O x C	1	471.85**	26.45	451.50**	46.53
Error (b)	10	21.54	26.89	29.67	35.04

**Indicates significance at the 0.01 level;

***Diffusible" refers to radioactivity eluted in 100 minutes into water; "exchangeable" refers to radioactivity eluted into IAA in 100 minutes above that obtained into water.

This decrease was associated with a significant increase in the capacity of the lazy plant tissue to retain radioactive IAA (Table 19).

Effect of gravity and temperature

The effect of metabolism on the release of IAA from tissue blocks was examined by elution at different temperatures. The tissue blocks for this experiment were the same as those for the transport experiment summarized in Table 15. At the termination of this experiment, the tissue blocks were eluted for 455 minutes. Half of the solutions were maintained at 5° C and the other half at 25° C. The amount of radioactivity eluted from the tissue blocks at a lower temperature would be expected to be less than at a higher temperature in the absence of active metabolic processes. In particular, the time-course of release at a lower temperature should be less by a constant factor dependent on the absolute temperature and the viscosity of water.

The parameters of the elution equation estimated from semi-logarithmic plots of the elution data of the experiment (Table 22) exhibited the same general order of magnitude as in the previous experiment (Table 20) except that the values

Table 22.--PARAMETERS OF THE EQUATION $E = 100 - Re^{-k_r t} - Se^{-k_s t}$ OBTAINED FROM THE DATA OF THE EXPERIMENT SUMMARIZED IN TABLE 23

Eluant	Orientation	Temperature	Parameter			
			R	k_r	S	k_s
		degrees C	percent	per hour	percent	per hour
Water		25	19.6	1.77	80.4	0.00583
		5	26.0	1.23	74.0	.00487
		25	20.0	2.77	80.0	.00582
		5	20.0	1.12	80.0	.0104
IAA		25	33.4	1.57	66.6	.0344
		5	30.5	1.37	69.5	.0270
		25	37.7	1.04	62.3	.0307
		5	26.5	1.17	73.5	.0215

of k_s were on the order of two- to four-fold lower. Elution in IAA resulted in several marked changes in the parameters of the system. The rate of elution, k_s, from the slowly turning-over component, S, was quadrupled in the presence of IAA; the average half-time of elution decreased from 103 to 24 hours. In contrast, the rate of elution, k_r, from the rapidly turning-over component, R, was slightly depressed in the presence of IAA. IAA in the eluting solution was also associated, on the average, with a transfer of about 10 percent of the radioactivity from S to R.

As before, the influence of various treatments on R can be inferred from the variation in amount of radioactive IAA eluted into water from the tissue blocks in 95 minutes. It was found that the amount eluted was not significantly affected by temperature (Table 23). It is therefore probable that active metabolic processes are involved in the release and maintenance of IAA. The finding that significantly less radioactivity is released into IAA at 5° C than at 25° C also suggests that at least a portion of the radioactive IAA is actively accumulated.

The significant interaction of orientation and temperature on the radioactive IAA released into water (Table 23) may be explained by the marked increase in k_r upon

Table 23.--THE EFFECT OF GRAVITY AND TEMPERATURE ON THE FORM OF RADIOACTIVE IAA IN TISSUE BLOCKS FROM NORMAL CORN STEMS

Conditions of the experiment as in Table 16

Orientation	Temperature	95 Minute "Diffusible" IAA***	95 Minute "Exchangeable" IAA***
	degrees C	percent	percent
	25	22.8	25.1
	5	25.4	13.6
	25	29.5	27.3
	5	19.8	12.4

Analyses of Variance

Source of Variation	d.f.	Mean Square "Diffusible"	Mean Square "Exchangeable"
Main plots			
Plants	2	38.26	118.81
Temperature (T)	1	74.90	1,040.16**
Error (a)	8	21.19	50.75
Sub-plots			
Orientation (O)	1	2.16	1.40
O x T	1	225.71*	16.34
Error (b)	10	32.26	25.52

**Indicates significance at the 0.01 level; *at the 0.05 level.

***"Diffusible" refers to radioactivity eluted in 95 minutes into water; "exchangeable" refers to radioactivity eluted into IAA in 95 minutes above that obtained into water.

horizontal placement at 25° C but not at 5° C (Table 22). It may also be observed in Table 22 that there was a transfer of radioactive IAA from S to R in polar-oriented tissue blocks as the temperature is depressed from 25° to 5° C. This transfer was not observed in polar-horizontal oriented tissue. The significant effect of temperature on radioactive IAA released into IAA solution above that released into water (Table 23) resulted chiefly from a decrease in both R and k_r with a decrease in temperature (Table 22).

The results of the previous experiments (Tables 20 to 23) suggested that there might be two components of radioactive IAA in S, one component requiring metabolic activity for maintenance, the other requiring none. Radioactive IAA was introduced into tissue blocks in the polar orientation for three hours in an experiment to test the existence of these two forms. Half of the tissue blocks were then suspended, still in the polar orientation, in washed cheesecloth in one liter of deionized water. The other half were suspended in 0.02 M potassium phosphate (pH6.0). One mg per liter Penicillin D was added to both to prevent bacterial action. The solutions were changed after eight and sixteen hours. The tissue blocks were removed from solution and blotted after a total period of 18 hours. Half of those

previously in water were placed in water at $1\pm1^{\circ}$ C and the remainder in 5×10^{-4} M IAA at $25\pm1^{\circ}$ C. Half of those previously in buffer solution were placed in buffer solution at $1\pm1^{\circ}$ C and the remainder in 5×10^{-4} M IAA in buffer solution at $25\pm1^{\circ}$ C. The tissue blocks were placed in fresh solutions after two hours and were removed after four hours.

After an elution period of 18 hours in water, radioactivity in the R compartment of tissue blocks should have been depleted to 0.002 percent of its original value. Placement of these tissue blocks at 25° C in IAA should remove both metabolically accumulated and physically exchangeable radioactive IAA. Placement of the blocks at 1° C in the solution containing no IAA should cause a release of metabolically accumulated radioactive IAA but not of physically exchangeable IAA. Buffer solutions were used in addition to deionized water to examine osmotic effects.

The results of the experiment presented in Table 24 indicated that the release of slowly eluted radioactive IAA is not an osmotic effect. Significantly more radioactivity was released at 25° C into IAA than into IAA-free solutions at 1° C. If this difference were attributable to changes in viscosity with the absolute temperature, then the rate of

Table 24.--RELEASE OF SLOWLY ELUTED RADIOACTIVE IAA FROM CORN NODE TISSUE BLOCKS INTO 5 x 10^{-4} M IAA AT 25° C AND IAA-FREE SOLUTIONS AT 1° C

Conditions of the Experiment

Plants: Third generation inbred normal (102-11, 106-6); 86 days old; shedding pollen
Nodes: 3-6 from apex
Tissue blocks: 4 x 4 x 4 mm
Time: 3 hours transport; 18 hours elution into water
IAA: 5 x 10^{-4} M, about 10,100 cpm per donor platelet

Eluant	Temperature Degrees C	Average Radioactivity Eluted per Block in 4 Hours
		cpm
IAA	25	292
Buffer-IAA	25	331
Water	1	175
Buffer	1	164

Analysis of Variance

Source of Variation	d.f.	Mean Square
Plants	5	18,423
Temperature (T)	1	121,453**
Eluant (water vs. buffer) (E)	1	1,211
T x E	1	3,833
Error	15	8,338

**Indicates significance at the 0.01 level.

elution at 1° C should differ by a constant factor of 2.11 from the rate of elution at 25° C. Examination of the time-course of release at these temperatures (Figure 15) clearly indicates no such constant difference.

Upper half versus lower half

In previous experiments examining the disposition of radioactivity in tissue blocks in the polar-horizontal orientation (Tables 20 and 21) half of the measurements were made on upper segments of the blocks. In this small number of tissue blocks no marked difference between upper and lower halves was observed in the form of the radioactive IAA. Therefore, a more extensive examination was made of possible differences in slowly eluted radioactive IAA between upper and lower halves of horizontally placed tissue blocks. Tissue blocks from nodes of normal and lazy plant stems were treated as outlined in the previous experiment (Table 24). After 20 hours soaking in water, the tissue blocks were bisected and oriented as either upper or lower halves in one of four solutions: deionized water, 10^{-3} M IAA, 10^{-3} M NP, or 5×10^{-4} M IAA plus 5×10^{-4} M NP. All solutions were maintained at $25 \pm 1°$ C and were adjusted to pH 7.0.

There was no significant difference in the amount of

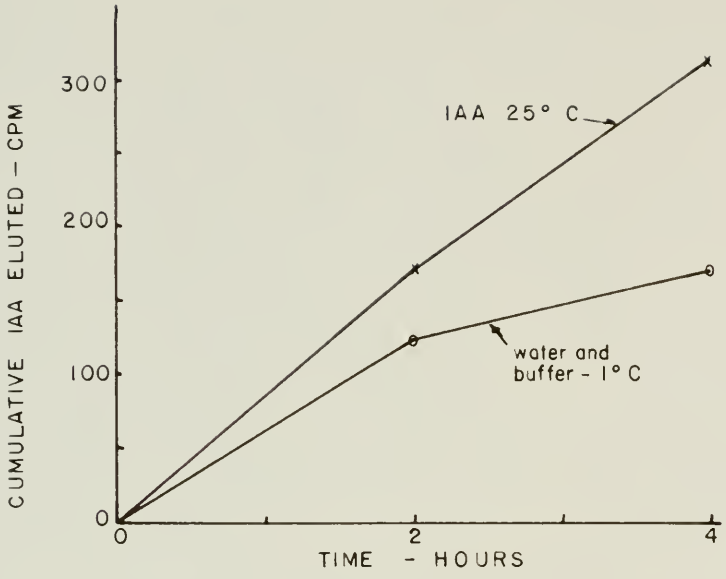


Fig. 15.--Time-course of release of radioactive IAA at 25^{o} C in 5 x 10^{-4} M IAA and at 1^{o} C in water or buffer from corn node tissue blocks previously soaked 18 hours.

slowly eluted radioactive IAA released from the upper and lower halves of horizontally placed tissue blocks of either lazy or normal plant stems (Table 25). Significantly more radioactive IAA was eluted into the IAA solution than into any other. The discovery that IAA was still released into water after 24 hours elution (Figure 16) supports the model of IAA existing in rapidly and slowly eluted pools. It can also be seen that measurably more IAA is eluted from tissue blocks from normal stems into NP than into water, whereas NP has no marked effect on the elution of IAA from tissue blocks from lazy stems.

Discussion

A quantitative examination of the disposition of IAA in tissue blocks in terms of "diffusible," "exchangeable," and "bound" components is not possible in the foregoing elution experiments since the continuing release of IAA from the tissue prevents precise identification of these components. The experimental results are better expressed in terms of two components; R, a rapidly eluted component, and S, a slowly eluted component. The amount of IAA eluted from the tissue, E, in time, t, is equal to $100 - Re^{-k_r t} - Se^{-k_s t}$.

R comprises about 20 to 40 percent of the total IAA and k_r corresponds to a half-time of 15 to 40 minutes. K_r

Table 25.--RELEASE OF RADIOACTIVE IAA FROM NODAL TISSUE BLOCKS FROM NORMAL AND LAZY CORN STEMS INTO DEIONIZED WATER, 10^{-3} M IAA, 10^{-3} M NP AND 5 x 10^{-4} M IAA PLUS 5 x 10^{-4} M NP AFTER 20 HOURS ELUTION IN WATER

Conditions of the Experiment

Plants: Third generation inbred (102-3 and 106-6); 93 days old; had shed pollen
Nodes: 3-6 from apex
Tissue blocks: 4 x 4 x 4 mm
Time: 3 hours transport; 20 hours diffusion; 4 hours elution
IAA: 5 x 10^{-4} M; about 10,100 cpm per donor platelet

Eluant	Radioactivity Eluted in 4 Hours			
	Normal		Lazy	
	Upper	Lower	Upper	Lower
	percent	percent	percent	percent
10^{-3} M IAA	15.7	16.1	15.2	16.6
10^{-3} M NP	7.8	7.1	6.8	5.6
5 x 10^{-3} M IAA + 5 x 10^{-3} M NP	12.2	12.2	11.8	13.6
Water	5.8	5.7	6.5	6.3

Analyses of Variance

Source of Variation	d.f.	Mean Square	
		Normal	Lazy
Main-plots			
Plants	4	21.95	14.97
Chemical (C)			
Water vs. NP (W)	1	13.61	0.16
IAA vs. IAA+NP (I)	1	68.45*	40.32*
(W) vs. (I)	1	549.82**	513.60**
Error (a)	12	8.38	5.96
Sub-plots			
Upper vs. lower (O)	1	0.61	1.36
O x C	3	1.09	3.97
Error (b)	16	2.23	4.19

**Indicates significance at the 0.01 level; *at the 0.05 level.

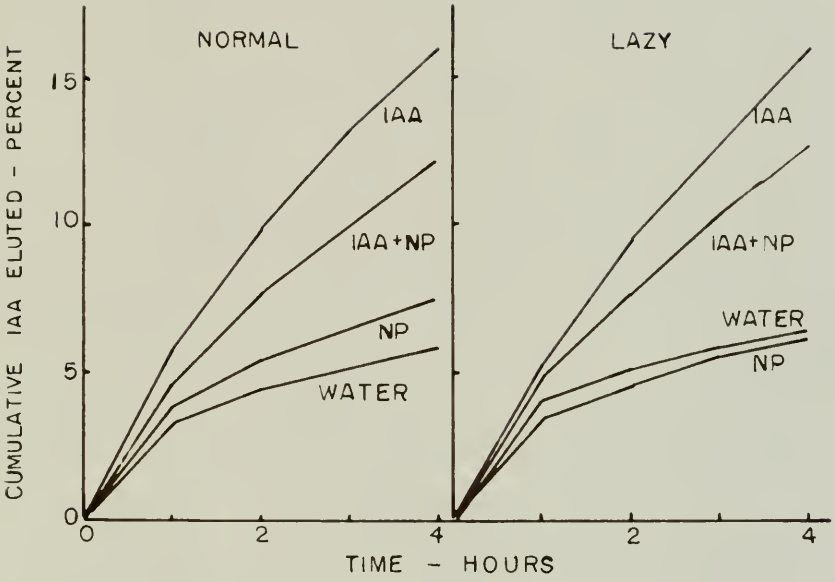


Fig. 16.--Time-course of release of radioactive IAA from tissue blocks from normal and lazy corn stems after 20 hours soaking in water.

is slightly depressed by the presence of IAA in the eluting solution or by low temperature, but not by NP. IAA in this rapidly eluted pool may be freely diffusible.

S comprises about 60 to 80 percent of the IAA in the tissue and k_s corresponds to a half-time of about 25 to 140 hours, depending on treatment. Elution of the S component of IAA at 1° C into water and at 25° C into IAA after essentially all of the R component of IAA is removed from the tissue (Figure 15) suggests the existence of two components of the S pool of IAA. Elution of radioactive IAA into IAA solution at 25° C should release both metabolically accumulated and physically exchangeable IAA. Elution into water at 1° C, however, should release only metabolically accumulated IAA. Elution experiments under similar conditions for longer periods of time should provide a better estimate of the proportion of these components in S. It is clear from these experiments, as well as previous studies (37, 69), that IAA exists in cells in at least three forms. Explanations of the effects of IAA only in terms of bound and diffusible forms fail to consider all of information available and perhaps lead to erroneous concepts. As pointed out by Galston and Purves (23), the existence of bound auxin is questionable. Sufficient method and theory are now

available to permit examination of the forms of auxin in cells in meaningful terms of physical-chemical associations.

The experiments reported in this section represent a beginning toward understanding the changes in the form of IAA in cells associated with the geotropic reaction and polar transport of IAA. Horizontal placement of normal corn stem tissue is associated with a transfer of IAA from the slowly eluted component, S, to the rapidly eluted component, R. In sharp contrast, lazy stem tissue or NP-infiltrated normal tissue, i.e., stem tissue incapable of responding to gravity, is associated with a transfer from pool R to pool S. Normal and lazy stem tissue are both capable of polar transport of IAA. Either horizontal placement of the tissue or NP treatment result in a reduction of polar transport and also in a reduction of k_s.

Clearly, the reorientation of tissue blocks from corn stems with respect to the direction of the force of gravity results in significant changes in the form of IAA in the tissue. A full explanation of the nature of these changes and their significance must await further study.

SUMMARY

The main purpose of this study was to examine the role of auxin transport in the geotropic reaction. The role of lateral transport in the reaction is implicit in the Cholodny-Went theory. Polar transport is also implicated in the reaction, since the zone of greatest geotropic sensitivity is not necessarily the zone of geotropic bending.

Study was limited to the negative orthogeotropic reaction of Zea mays L. (corn). This plant was chosen as experimental material because of the availability of a single gene ageotropic mutant "lazy," which would be expected to possess a single primary physiological deficiency. Radioactive indole acetic acid (IAA) and recently discovered selective inhibitors of the geotropic reaction were employed in conjunction with the methods of physiological genetics to examine this reaction. The radioactivity was used to measure transport and binding of the hormone; and the inhibitors used to block selectively the geotropic reaction without inhibiting growth.

IAA transport was measured in tissue blocks from corn stems by applying IAA 2-C^{14} in agar to one face of a tissue

block and measuring the appearance of radioactivity in plain agar on an opposite face of the block. Transport in the tissue blocks was studied as influenced by chemical inhibitors of geotropism, tissue orientation, and the morphological side of application of IAA. The form of IAA in tissue blocks was studied by eluting the radioactive IAA from them into various solutions.

The effect of chemical inhibitors on the geotropic reaction of corn stems was assayed using isolated nodal stem segments. Segments were infiltrated, by vacuum, with a solution to be tested and then placed horizontally by inserting their bases in wet plastic foam. The angle of geotropic bend of a segment was determined after 96 hours.

The various types of experiments were conducted on stem tissue from both normal and lazy corn plants.

The results of this study justify the following statements:

1. IAA exists in at least three forms in corn stems: diffusible, exchangeable, and actively accumulated. Diffusible IAA is rapidly eluted from tissue, whereas exchangeable and actively accumulated IAA are slowly eluted. No evidence of irreversibly bound IAA was observed.

2. N-1-naphthylphthalamic acid (NP) and

2,5-dinitrophenol inhibit both polar transport of IAA and the geotropic reaction of corn stems.

3. Lateral transport of IAA in corn stem tissue blocks is apparently a passive process, slower than the rate of diffusion of IAA through agar.

4. No effect of gravity on lateral transport could be observed. It is suggested that processes other than lateral transport cause the unequal distribution of diffusible auxin resulting from geotropic stimulation.

5. Horizontal placement of normal corn stem tissue results in marked reduction in polar transport of IAA, in redistribution of IAA among its various forms, and in changes in their rates of elution.

6. Horizontal placement of NP-infiltrated normal stem tissue results in patterns of redistribution significantly different from those of normal tissue.

7. The mature stem of lazy corn plants, although capable of perceiving gravity, is incapable of responding normally to gravity.

8. Horizontal placement of lazy corn stem tissue results in marked reduction in polar transport of IAA and changes in the rates of elution of the forms of IAA similar to those observed in normal tissue. Redistribution of IAA

among its various forms also results from horizontal placement of lazy corn stem tissue but these changes are similar to those observed upon reorientation of NP-infiltrated tissue.

9. Geotropic perception and polar transport of IAA may occur in the phloem.

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APPENDIX

APPENDIX

Dr. A. L. Koch made the following derivation to examine the effect of accumulation of a compound within a cell on the transport of that compound through the cell. Considering a single row of cells, let C=cell volume; s=interstitial volume; V=maximum velocity of pumping into the cell; H=the permeability constant with the dimensions of cm^3 per unit time; I=the internal concentration of a solute within a cell; and E=the concentration in the interstitial area.

If a cell has equal capacity on all sides to pump the solute, then

$$(1) \quad C\frac{dIn}{dt} = \frac{VEn-1}{K+En-1} + \frac{VEn}{K+En} - 2HIn + HEn + HEn-1$$

and $$(2) \quad s\frac{dEn}{dt} = HIn + HIn+1 - 2HEn - \frac{2VEn}{K+En}.$$

In the steady state $\frac{sdEn}{dt}$ approaches zero. Rearranging equation (2) and making the appropriate substitutions in equation (1)

$$C\frac{dIn}{dt} = \frac{HIn + HIn+1}{2} + \frac{HIn-1 + HIn}{2} - 2HIn$$

and finally $$\frac{dIn}{dt} = \frac{H}{2C}\Delta^2 I$$

BIOGRAPHICAL SKETCH

Theodore Waage Holmsen was born March 1, 1930, in Teaneck, New Jersey. He was graduated from Flemington High School in June, 1948. After two years of undergraduate study, he entered the United States Naval Flight Training School, Pensacola, Florida. In April, 1954, he was commissioned as a pilot in the United States Marine Corps. Upon release from active duty, he entered Rutgers University, receiving a Bachelor of Science degree in October, 1957. In 1957 he enrolled in the Graduate School of Rutgers University and was appointed a research assistant in the Department of Horticulture. He received the degree of Master of Science in October, 1958. In September, 1958, he enrolled in the Graduate School of the University of Florida. He worked as a research assistant in the Department of Botany until June, 1960. From June, 1960, until the present time he has been a General Biological Supply House Scholar pursuing work toward the degree Doctor of Philosophy.

Theodore Waage Holmsen is married to the former Claire Blanche Caka and is the father of three children. He is a member of the Botanical Society of America, the American Society of Plant Physiologists, Phi Beta Kappa, Sigma Xi, Phi Sigma Society, and Gamma Sigma Delta.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1961

Dean, College of Agriculture

Dean, Graduate School

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