MOVEMENT OF INDOLE-3-ACETIC ACID FROM THE ENDOSPERM TO THE SHOOT OF ZEA MAYS L.

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ABSTRACT

MOVEMENT OF INDOLE-3-ACETIC ACID FROM THE ENDOSPERM TO THE SHOOT OF *ZEA MAYS* L.

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A large body of literature suggests that a growth hormone precursor moves from the seed to the seedling tip, is there converted to an active hormone, and is then transported downwards to control the rate of extension growth (26). The structures and the concentrations of all of the indolylic compounds that occur in the seeds of corn (*Zea Mays* L.) are now known. Thus, it should be possible to determine which, if any, of the indolylic compounds of the seed can be transported to the seedling in, seemingly, significant amounts. Of interest would be the transport of tryptophan, free indole-3-acetic acid (IAA), and the esters of IAA, which comprise 95% of the IAA compounds. In this work I have shown that, (1) IAA can move from the seed to the shoot, (2) 90% of the transported IAA has been metabolized into compounds other than IAA *en route*, and (3) some of the IAA that has moved into the shoot has been esterified. With certain assumptions concerning IAA-flux and metabolism in the shoot, it can be concluded that the

amount of IAA transported and remaining as IAA in the shoot is inadequate to sustain growth.

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INTRODUCTION

It has been suggested that a growth hormone or a hormone precursor is moved from the seed to the shoot in corn (26). The identity of the transported compound is not known, but could possibly be an IAA-ester, tryptophan, or free IAA. The content of IAA in sweet corn is between 70 to 90 mg of IAA per kilogram of dry kernels (24). In 1941, Avery, Berger, and Shalucha (1) found that more IAA could be extracted from corn kernels after hydrolysis with alkali. Three years later, Berger and Avery (3) showed that kernels of corn contained an auxin precursor that produced IAA on alkaline hydrolysis. Subsequent work has shown that these alkali-labile IAA compounds consist of IAA-myo-inositols, IAA-myo-inositol arabinosides, IAA-myo-inositol galactosides, IAA-glucose, an IAA-cellulosic glucan with the glucan chain of variable length, and small amounts of free IAA (15, 23, 8, 18). Ueda (24) found about a 1% per hour decrease in concentration of all the IAA-esters in corn kernels during germination.

This work begins an examination of which compounds, free IAA or IAA derivatives, can move from the seed to the shoot where growth is occurring. By knowing the rate of movement it may be possible to decide which compounds move at a sufficient rate to account for the IAA and IAA-esters in the shoot.

REVIEW OF LITERATURE

Cholodney (5) found that a growth hormone diffused out of dehusked *Avena* seeds when the seeds were put in hot water or ethanol. When coleoptile tips were placed on one side of a decapitated coleoptile, a bend occurred which lasted for less than one hour. A piece of the endosperm placed on a coleoptile produced a curvature which lasted for 3 to 4 hours. Cholodny suggested there was a continual formation of growth hormone in the endosperm and that the growth hormone moved from the seed to the shoot.

Skoog (21) decapitated oat seedlings, removed the primary leaves, and placed agar blocks on the coleoptile stumps. He then tested the blocks using the deseeded *Avena* test and found no auxin in the agar blocks after 5 to 6 hr, but he did detect auxin when the block had been left on the stump 10 to 20 hr. Skoog also found an auxin precursor that diffuses out of the tip of oat coleoptile sections but not out of the base. He suggested this could be an IAA precursor which is moved from the seed to the shoot. Skoog found the seed to be necessary for regeneration of the physiological tip in decapitated coleoptiles.

Thimann (22) applied IAA in lanolin paste to the scutellum of _Avena_ seedlings and observed a slight decrease in growth of the coleoptile due to poorer root development. When seedlings were placed with the roots in an IAA solution neither an inhibition nor promotion of growth of the coleoptiles was observed.

Whitehouse and Zalik (27) found that a radioactive compound with the R_f of IAA could be extracted from corn shoots after ^{14}C-IAA had been injected into the endosperm. They also found minor radioactive peaks at lower R_f's. No vascular strands were found in the endosperm but vascular tissue was present in the scutellum. When roots of seedlings were placed in a vital stain no dye entered the endosperm, but a vascular strand in the scutellum branched and entered the interface between the scutellum and endosperm. Two vascular bundles in the coleoptile were stained. Dye injected into the endosperm did not reach the xylem in the scutellum but did enter between the endosperm and scutellum. Using _Phaseolus coccineus_, Whitehouse and Zalik destroyed living tissue in a region of the stem with a hot glass rod. The movement of ^{14}C-IAA up the stem after injection into cotyledons was drastically reduced by searing the cotyledons with the glass rod, but die still moved up the stems. This indicates that translocation does not occur in the xylem. Hall _et al_. (12) identified IAA in the phloem by mass spectrometry and in root pressure saps of _Ricinus communis_. The authors suggest IAA may move more freely in the non-polar phloem.

Sheldrake (20) found IAA and alkali-labile IAA compounds in the xylem sap of *Zea* when the coleoptile tip had been cut off. ^{14}C-IAA injected into *Avena* endosperm can be detected in guttation fluid. Sheldrake also sites the autonomous curvature of *Avena* coleoptiles as circumstantial evidence for acropetal movement of auxin in the xylem. This curvature correlates with the asymmetry of the xylem strands at the coleoptile tip. IAA in the phloem could not produce this curvature.

A literature review of auxin transport has been published by Goldsmith (9). She states that endogenous and applied auxin move predominantly in the basipetal direction and that this movement depends on living cells. Characteristics of the polar movement are that the velocity is independent of donor concentration and length of section, and that active metabolism is necessary. Goldsmith (10) observed the basipetal movement of IAA in 10 mm sections of oat coleoptiles under anaerobic conditions and found the amount of IAA in the tissue to be significantly decreased within one half hour after anaerobic conditions were begun. In contrast, a significant difference in the amount of IAA that had moved acropetally into the tissue under anaerobic conditions was not observed for two hours. Goldsmith also found that only 10% of the IAA that had been moved acropetrally in aerobic conditions could be rinsed out with large volumes of water while all of the IAA that had been taken up under anaerobic conditions could be rinsed out.

She found that anaerobic uptake fit diffusion theory with a diffusion constant of approximately 1 x 10^{-4} mm^2/sec. Under aerobic conditions, diffusion theory does not hold for movement toward the tip because of immobilization of IAA. This immobilization of IAA explains why basal uptake occurs against a concentration gradient and why the first mm of the section has a higher concentration of IAA then the donor. Goldsmith (11) also found that basipetal transport recycled IAA that had been moved toward the tip.

McCready (17) did a series of experiments with 5 mm sections of young, rapidly elongating petioles of the primary leaves of *Phaseolus vulgaris* L. to determine if base directed and tip directed movement occurred by the same mechanism. He found that low temperature effected the flux of IAA towards the base much more than the flux towards the tip. Inhibitors also had different effects on movement towards the base and towards the tip. Napthylphtalamic acid (NPA) decreased base directed transport until it was equal to tip directed movement. Movement toward the tip was not effected by NPA. Fluorenolcarboxylic acid; 2,3,5-triiodobenzoic acid; and sodium azide decreased flux toward the base until it was equal to flux toward the tip. At high concentrations of inhibitor, tip directed movement was slightly increased. This could be due to toxic action of the inhibitors which might break down permeability barriers. McCready found no evidence for the existence of an active component in the movement of IAA towards the tip and that the major part of movement towards the tip has the characteristics of passive diffusion.

It should be noted that in none of the above described work was an isolation procedure for IAA used that has been shown to yield pure IAA thus proving that the radioactivity was in IAA. The method used for IAA isolation in this work, as described below, has been shown to yield pure IAA from *Zea* (2).

MATERIALS AND METHODS

Combustion of tissue for determining total radioactivity was done on a Packard Model 306 Tri-Carb Sample Oxidizer. Gas-liquid chromatography was done on a Varian 2740 gas chromatograph with a flame ionization detector and nitrogen as the carrier gas. Ultraviolet spectra were recorded with a Cary 15 spectrophotometer. Bray's solution (4) was used to determine radioactivity with a Packard Tri-Carb model 3003 liquid scintillation counter for ^{14}C and a Beckman CPM-100 Liquid Scintillation System for ^{3}H.

Materials used were from the following sources: 2-^{14}C-IAA (specific activity 23.5 mCi/mmol) : Schwarz/Mann; 5-^{3}H-IAA (specific activity 23.5 Ci/mmol) : CEA France, obtained through Dr. M. H. Goldsmith at Yale University; IAA and indole-3-butyric acid : Calbiochem; DEAE-cellulose : Sigma; Sephadex LH-20 : Pharmacia; Silica Gel G plates, Merck Darmstadt : Brinkman; 5% SP-2401 on Supelcoport : Supelco; bis (trimethylsilyl) trifluoroacetamide : Regis; Stowells Evergreen Hybrid Corn 1974 harvest : Ferry Morse Seed Co.

Plant Material

Corn kernels were surface sterilized, soaked in aerated water for 16 hr, then placed in a horizontal row across a paper towel. The towel was then rolled, secured with tape and placed edge up in a 5 liter beaker containing 400 ml of water. When the beaker was full of towels it was covered with plastic wrap and placed in a dark room at 25 C. Four-day-old seedlings were used for the transport studies. The shoots were between 1.5 and 3.0 cm long.

Application of Radioactive IAA

About one-half of the seed was cut off from each seedling under a green safelight leaving the embryo, scutellum and about 2 mm of the endosperm intact. Five μl of radioactive IAA in 50% ethanol was applied to the cut endosperm surface, corresponding to 7.2 ng and 2100 dpm when ^{14}C-IAA was used and 7.6 ng and 2,210,500 dpm when ^{3}H-IAA was used. A group of five seedlings, with their cut surfaces facing upward, were placed in a 9 cm petri dish kept humid with moist filter paper. The seedlings were incubated at 25 C for the indicated times, then the shoots, and in some cases the roots, were cut from the kernel and frozen at -20 C until used for oxidation or extraction.

Since the endosperm liquifies during germination, this method of application does not require that the radioactive compound permeate any membrane barriers other than those which any seedling-endosperm compound would have to permeate.

Tissue Combustion

Shoots and roots, in groups of ten, were combusted in the oxidizer, the resultant CO_2 trapped in an organic base and the radioactivity counted in a liquid scintillation counter.

Reisolation of Free ^{3}H-IAA

The isolation procedure adopted is cumbersome and requires almost one week for a single assay. Its advantage is that it has previously been shown to yield pure IAA from seedlings of *Zea*. Since many IAA adducts and oxidation products have TLC and paper chromatographic mobilities similar to IAA, a rigorous purification must be employed.

Sixty shoots were ground with a mortar and pestle in enough acetone to make the solution 70% acetone-water. The homogenate was extracted two more times with 70% acetone and the extracts combined and filtered. Five hundred µl of IAA and 500 µl of indole-3-butyric acid were added. The filtrate was concentrated to 5 ml on a flash evaporator in a water bath at 50 C. The pH was adjusted to 2.5 and the sample extracted three times with 10 ml of diethyl ether each time. The ether was then extracted three times with 5 ml of 1 M $NaHCO_3$. After readjusting the pH to 2.5 the $NaHCO_3$ was extracted three times with diethyl ether. The ether was dried and the sample taken up in 1 ml of $CHCl_3$. For the last experiment the concentrated filtrate at pH 2.5 was extracted into $CHCl_3$ three times instead of the ether, $NaHCO_3$, and ether extractions.

DEAE-cellulose column chromatography, Sephadex LH-20 chromatography, thin layer chromatography, silylation and gas-liquid chromatography, and UV spectrometry were carried out as described by Bandurski and Schultz (2). The DEAE-cellulose column was prepared as described by Rouser *et al.* (19) for lipids, except that initially the column had to be washed with 100 ml of $CH_3OH/CH_3COOH/(C_2H_5)_3N$ (20:4:1), then regenerated before IAA would bind to it. The sample, in one ml of $CHCl_3$, was applied to the column and the column eluted with; (a) 200 ml of $CHCl_3$; (b) 200 ml of $CHCl_3/CH_3OH$ (9:1); (c) 400 ml of $CHCl_3/CH_3OH/CH_3COOH$ (7:3:0.01%); (d) 500 ml of $CHCl_3/CH_3OH/CH_3COOH$ (7:3:1%). Percentages are v/v. The fractions containing IAA were determined by UV absorption at 282 nm. The IAA was eluted between 350 and 450 ml of solvent *d*. The fractions containing IAA were pooled and evaporated to dryness. The column was regenerated with; (a) 200 ml of CH_3COOH; (b) 400 ml of CH_3OH; (c) 200 ml of $CH_3OH/CHCl_3$ (1:1); (d) 300 ml $CHCl_3$.

The sample from the DEAE-cellulose column was dissolved in 1.0 ml of 50% (v/v) ethanol-water and applied to a 22 x 1.8 cm column of Sephadex LH-20 that had been washed with 50% ethanol. The sample was eluted with 50% ethanol at a flow rate of 3.5 to 4.0 ml/hr. Using UV absorption, IAA was found between 80 and 100 ml. The fractions containing IAA were pooled and evaporated to dryness. The column was regenerated with large volumes of 50% ethanol-water.

The sample from the LH-20 column was dissolved in 200 ul of 50% ethanol and applied in a 10 cm streak across a 20 cm X 20 cm thin layer silica gel G chromatography plate. On each side of the sample streak, 1 cm streaks of the sample and guide spots of IAA were applied. The plate was run in a solvent consisting of benzene-acetone-pyridine 60:39:1. The ends of the plate, where the 1 cm streaks and the guide spots had been applied, were cut off, and sprayed with a color reagent to determine the migration of IAA in the sample. The region where IAA was present was scraped from the plate and the silica gel was washed three times with 5 ml of 50% ethanol-water. Each time the silica gel was sedimented by centrifugation for 10 min at about 500 x g. The ethanol extracts were combined, filtered through Whatman No. 42 filter paper, and taken to dryness. To remove the small residue of silica gel still present the sample was dissolved in 200 ul of 50% ethanol, transfered to a clean drying flask and redried.

The sample was then redissolved in 200 ul of 50% ethanol, transfered to a serum vial, dried under nitrogen at 70 C, and sealed with a rubber top. Silylation was accomplished by adding 20 µl of bis(trimethylsilyl)-trifluoroacetamide and 10 µl of redistilled pyridine to the sealed vial with a syringe. The sample was kept at 45 C for 15 minutes. Gas-liquid chromatography was on 5% SP-2401 on 100/120 mesh Supelcoport in a 1.8 m x 6 mm glass column at 165 C, with 40 ml/min of nitrogen as carrier gas.

IAA was first injected into the column to determine its retention time which was typically 15 minutes. A few μl of the sample was then injected to determine the sample profile. The rest of the sample was then injected 7 μl at a time and the IAA was collected by extinguishing the hydrogen flame and slipping a glass tube over the detector outlet. The IAA which condensed on the glass tube was then washed into a quartz cuvette with 1 ml of redistilled methanol.

The UV spectrum of the sample was recorded. The 282 nm and the 225 nm peaks were used to determine the amount of IAA present. Absorption by p-coumaric acid was corrected for by multiplying the sample absorbance at 330 nm by 4.0 or 3.3 and subtracting these values from the absorbance at 282 and 225 nm, respectively. The molar extinction coefficient for IAA is 6060 at 282 nm and 33,200 at 225 nm.

One half ml of the sample was added to 5 ml of Bray's solution and counted for 100 minutes in a liquid scintillation counter.

A correction for the amount of radioactive IAA lost in the purification procedure was made by using a reverse of the isotope dilution assay. The recovery of ^{3}H-IAA was assumed to equal the recovery of the 500 μg of cold carrier IAA added at the beginning of the purification. The total radioactivity in the corn shoots was calculated from the equation:

$$\frac{\text{carrier IAA added}}{\text{carrier IAA recovered}} \times \begin{pmatrix}\text{radioactivity of} \\ \text{recovered sample}\end{pmatrix} = \begin{matrix}\text{radioactivity} \\ \text{in shoots}\end{matrix}$$

<u>Reisolation of Free Plus Alkali-Labile ^{3}H-IAA</u>

The filtered acetone extract from 120 shoots to which had been added 1 mg of IAA and 1 mg of indole-3-butyric acid was divided into two equal aliquots and taken to dryness. One aliquot was taken up in 5 ml of 1 N NaOH and allowed to stand for one hour in a 100 C oven, then neutralized with H_2SO_4. The other aliquot was taken up in 1 M Na_2SO_4. ^{3}H-IAA was reisolated from both aliquots, after adjustment of the pH to 2.5, as described for free IAA above. An additional determination of the amount of IAA recovered was made with a colorimetric assay [(7) and personal communication from Dr. Ehmann].

RESULTS

Total Radioactivity Moved to Shoot and Root

The CO_2 derived from complete combustion of the shoot or root contained radioactivity one hour after the labeled IAA had been applied to the endosperm. Figure 1 shows the amount of radioactivity found in the shoot and root as a function of incubation time. Each point on the graph is the average radioactivity per shoot or root obtained from counting two groups of 10 shoots or two groups of ten roots. After 8 hr, about 30 cpm were found in each shoot. At a counting efficiency of 77%, this represents 1.9% of the applied radioactivity. If all the radioactivity were still in IAA, this would correspond to 1.4×10^{-10} g IAA/shoot.

^{3}H-IAA Moved to Shoot

The results of experiments in which ^{3}H-IAA was reisolated from the shoot are shown in Table 1. The cpm data were corrected for recovery of the 500 μg IAA added to the acetone extract and for a 33% counting efficiency for ^{3}H. The average amount of IAA that moved from the

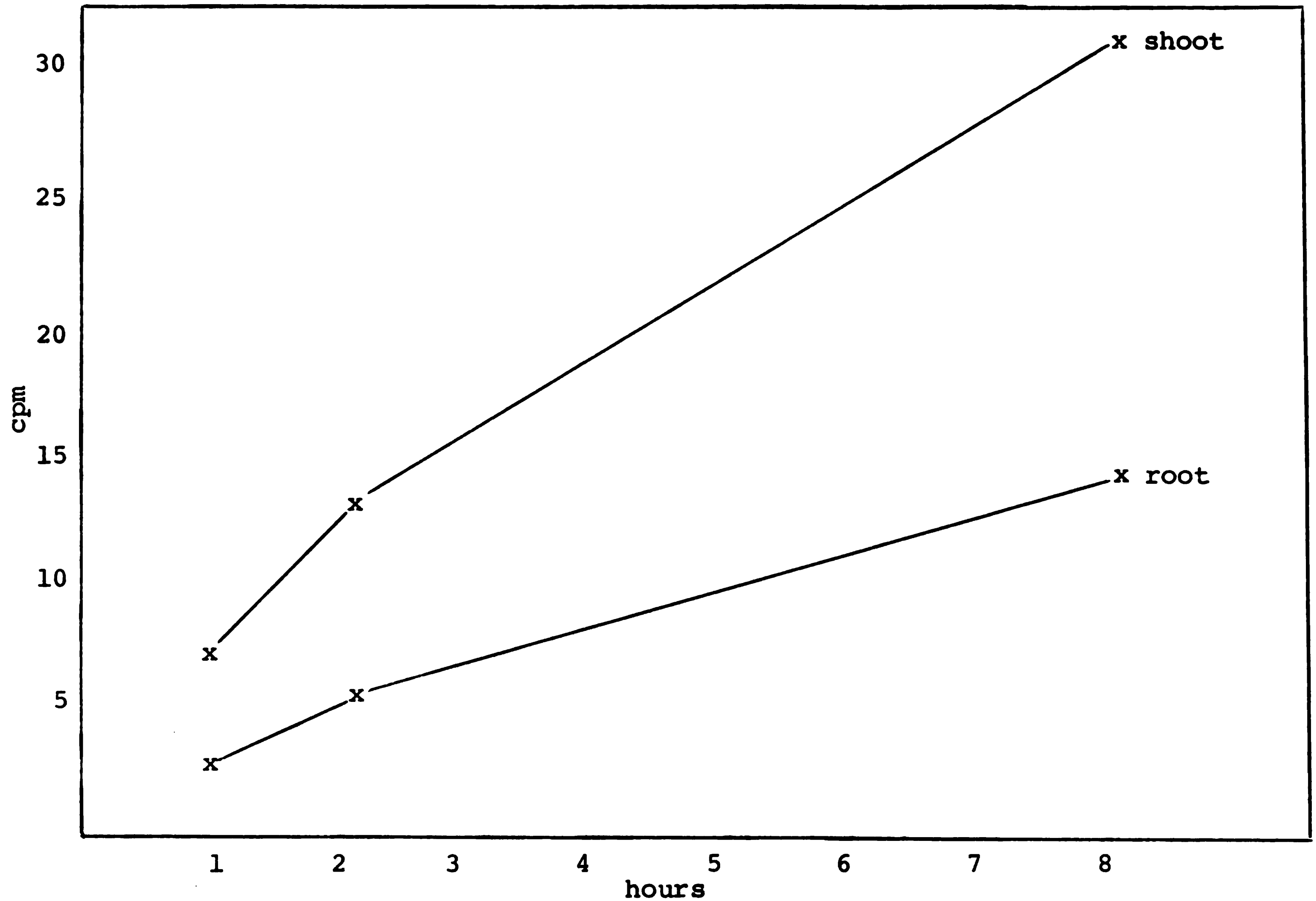

Figure 1. Radioactivity found in the shoot and root of a corn seedling after 2100 dpm were applied to the endosperm.

Table 1.--Reisolation of ^{3}H-IAA from corn shoots after application to the endosperm.

Experiment	Treatment of Acetone Extract	Percent Recovery of Added IAA Determined by		cpm for Half of Sample	(Corrected)	g IAA per Shoot x 10^{13}
		282 Peak	Colorimetry			
1*	not hydrolized	4.4		203	27,936	16
2*	not hydrolized	1.7		55	19,186	11
3	not hydrolized	1.5	1.9	25	10,273	5.8
	hydrolized	2.4	2.3	68	17,000	9.6
4	not hydrolized	4.0	3.1	104	15,600	8.8
	hydrolized	4.9	4.0	165	20,122	11
5	not hydrolized	3.6	2.8	78	14,338	8
	hydrolized	1.4	0.9	109	50,453	28

*In experiments 1 and 2, the acetone extract was not divided into two aliquots, one with and one without alkaline hydrolysis, so these experiments do not show the amounts of labeled IAA incorporated into the ester fraction.

endosperm was 1.04 x 10^{-12} g/shoot. Some of the ^{3}H-IAA extracted from the shoot had been incorporated into an alkali-labile complex accounting for 40%, 20%, and 70% of the IAA in the three hydrolized samples. After correcting for equilibration of the ^{3}H-IAA with the endogenous pool of free IAA in the endosperm (71.9 μg/100 g, personal communication from Mr. Jerry Cohen) a value of 1.6 x 10^{-11} g/shoot is obtained. If the applied ^{3}H-IAA also equilibrated with the endogenous bound IAA which Ueda (24) estimated at 6 mg/kg in four-day-old seedlings the amount of IAA moved to the shoot would be 2.1 x 10^{-10} g/shoot. This is, however, unlikely since Kopcewicz, et al., (14) have shown that the ester pool is only slowly labled with ^{14}C-IAA by germinating kernels. Equilibration of the ^{3}H-IAA with about 8% of the ester IAA in the endosperm would be more likely since Ueda and Bandurski (24) have shown that 1% of the esterified IAA is lost each hour for the first 96 hours of germination. This assumes that the pathway for loss is ester IAA→free IAA→oxidized IAA. If ^{3}H-IAA equilibrates with 8% of the ester pool the amount of IAA moved to the shoot would be 3.0 x 10^{-11} g/shoot.

DISCUSSION

Four, and possibly five, conclusions can be drawn from this work: First, IAA applied to corn endosperm can be reisolated from the shoot. This is the first time this has been demonstrated unambiguously using an isolation procedure that is known to yield pure IAA. Secondly, 90% of the radioactivity of the labeled IAA applied to the endosperm is no longer IAA by the time it has reached the shoot. Thirdly, labeled IAA becomes esterified either in the endosperm or in the shoot. Thus, the transport form of IAA cannot be determined from these experiments. ^{3}H-IAA could have been esterified in the seed and moved from the endosperm to the shoot as an ester, or free ^{3}H-IAA could have been esterified in the shoot. Fourth, a diffusion constant can be calculated for IAA moving through the seedling. Lastly, if certain assumptions are made concerning the rate of flux of IAA, that is rate of formation and destruction of IAA, then a tentative conclusion concerning the amount of IAA transported can be made.

Assuming my experimental arrangement to be analogous to solute diffusion from a large reservoir into an infinitely long capillary where the initial concentration is zero, a diffusion constant (D) can be calaculated with the equation:

$$\frac{C}{C_o} = 1 - \frac{2}{\pi} \int_o^{\frac{X}{2\sqrt{Dt}}} e^{\frac{-X^2}{4DT}} dX = \text{erfc} \frac{X}{2\sqrt{Dt}}$$

where C_o is the concentration at the source, C is the concentration in the section, and X is the distance between the source and the section (10). In calculating the diffusion constant I assume that the radioactivity applied has spread out into a two dimensional volumeless plane and that the radioactivity in the shoot is also in a two dimensional volumeless plane at the surface where the shoot was cut. Ignoring the endogenous IAA in the seedling, this allows me to let C/C_o equal the radioactivity in the shoot over the radioactivity applied. The distance (X) from the surface where the radioactivity was applied to the place where the shoot was cut is approximately 1 cm. The error function complement (erfc) can be found in tables (6).

When $\frac{C}{C_o} = \frac{30}{2100} = .01428$

$$\frac{x}{2\sqrt{Dt}} = 1.7 \text{ (from table)}$$

$$D = 3 \times 10^{-6} \text{ cm}^2/\text{sec}$$

The diffusion constant for radioactivity that moves into the seedling when ^{14}C-IAA is applied to the endosperm is 3×10^{-4} mm^2/sec.

However, D is smaller when ^{3}H-IAA was applied to the endosperm and pure IAA isolated from the seedling.

$$\text{When } \frac{C}{C_o} = \frac{306}{2,210,500} = .000138$$

$$\frac{x}{2\sqrt{Dt}} = 2.7$$

$$D = 1.2 \times 10^{-6}\ \text{cm}^2/\text{sec}$$

The diffusion constant for ^{3}H-IAA is 1.2×10^{-4} mm^2/sec. This value for ^{3}H-IAA is comparable to the diffusion constant of 1.26×10^{-4} mm^2/sec calculated by Goldsmith (10) for IAA during basal uptake in oat coleoptiles under anaerobic conditions. These diffusion constants are well below the value of 6.9×10^{-4} mm^2/sec found by Larsen (16) for the diffusion of IAA through agar.

Dr. Jerry Pollack has found that the principle barrier to diffusion of Xe^{133} through frog skins and toad bladders is the inter- and intra-cellular water (personal communication from Dr. Pollack). By using the diffusion constant of Xe through water divided by the thickness of frog skin or toad bladder he calculates 2.4×10^{-3}mm/sec and 12×10^{-3} mm/sec, respectively. These values correspond to the measured diffusion constants of 3.9×10^{-3} mm/sec and 7.4×10^{-3} mm/sec for frog skin and toad bladder. The principle barrier to IAA diffusion may also be water and IAA is expected to diffuse in a manner similar to Xe except that the negative charge on IAA and its higher molecular weight probably make IAA diffusion slower than diffusion of Xe. The diffusion constant of Xe through water is $12 \times 10^{-4} mm^2/sec$ (25). This value is also larger than the diffusion constant I found for IAA. Thus, simple diffusion can account for the movement of IAA from the seed to the shoot seen in these experiments.

Went and Thimann (26) showed that maximal curvature of oat coleoptiles occurs with as little as 0.2 mg/l of IAA in a 10 mm^3 agar block when the angle of curvature was measured 6 hr after application of the block. It was shown that only 15% of the IAA in a block this size enters the plant. This corresponds to 3×10^{-10} g IAA per plant for optimal curvature. Skoog (21) found that 1.5×10^{-12} g IAA/shoot was needed for a 1° curvature. It should be emphasized that there are insufficient data to estimate the rate at which IAA is being made and destroyed (IAA-flux) and in the absence of such data it is impossible to calculate the amount of IAA being transported. Some known routes of IAA formation and destruction are:

$$\begin{array}{c} \text{tryptophan} \rightarrow \text{IAA} \rightarrow \text{IAA metabolized} \\ \uparrow \downarrow \quad \nearrow \\ \text{IAA-esters} \ ? \end{array}$$

The release of free IAA from IAA-esters can be deduced from experiments with vegetative tissue (13). The formation of IAA-esters using free IAA is a known reaction (14). It is not known if the IAA in an IAA-ester can be directly metabolized. As a first approximation and with care to indicate the assumptions made, it is desirable to make the best estimate of the amount of IAA being transported. I am assuming (1) that IAA is not being made in the seedlings from tryptophan or any other precursor, (2) that the IAA-esters which disappeared during germination (Ueda and Bandurski (24) observed that 1% of the IAA esters disappeared per hour during the first 96 hours of germination) are first hydrolized to yield free IAA which is then used in growth or destroyed, (3) that none of the radioactive IAA-metabolites (that is the radioactivity in the shoot that could not be accounted for as IAA or IAA-esters) were a result of IAA destruction after the hormone had been utilized to promote growth. Applied IAA would not equiliberate with all the IAA in the seed (14). A value of 1.6×10^{-11} g of IAA per shoot was obtained from my experiments when the specific activity of the IAA is corrected for dilution with the free IAA pool and 3×10^{-11} g of IAA per shoot if the IAA dilution is with the free IAA plus 8% of the ester-IAA. This value is an upper limit since it assumes no ester destruction without prior hydrolysis to yield free IAA. These calculations are summarized in Table 2. Thus, the IAA I observed moving to the shoot is too low by a factor of 10 to be sufficient to promote optimal growth as determined by Went's value for optimal curvature.

The average growth in a shoot during the 8 hr period was 0.036 g per shoot. The growth is predominantly an

Table 2.--IAA transport from endosperm to shoot.

Basis for Calculation	Amount of IAA Transported into Shoot
Assuming no dilution of IAA applied to endosperm	1.0×10^{-12} g/shoot
Assuming dilution only with free IAA in the endosperm	1.6×10^{-11} g/shoot
Assuming dilution with free IAA plus 8% of the IAA esters in the endosperm	3×10^{-11} g/shoot
Amount of IAA required to give maximum curvature (Went and Thimann)	3×10^{-10} g/shoot
Amount of IAA that must move from the endosperm to the shoot tissue to maintain a concentration of 2×10^{-6}M IAA in the shoot	1.3×10^{-8} g/shoot

increase in water content. IAA from the seed would presumably be used to keep the concentration of IAA in the shoot at the levels found in seedlings. Using the value obtained, assuming the ^{3}H-IAA was diluted only by the free IAA in the kernel, 1.6×10^{-11} g in 0.036 ml water gives a concentration of 2.5×10^{-9} M IAA. Assuming the applied ^{3}H-IAA was diluted in the free plus 8% of the ester IAA, a concentration of 4.8×10^{-9} M IAA is calculated. These values are considerably below 2×10^{-6} M IAA found in shoots of five-day-old corn seedlings (2). Thus, whether the movement of IAA observed in these experiments is sufficient to be of physiological significance seems doubtful.

I would like to note that while 1.9% of the applied radioactivity was recovered from shoots with complete oxidation of the tissue, only 0.014% of the applied radioactivity could be re-extracted as IAA. Thus, metabolism of the radioactive IAA to things other than IAA esters accounts for the majority of the radioactivity that had moved to the shoot.

Further studies, with labeled tryptophan and labeled IAA-esters, are required before a rational decision concerning the identity of the diffusable auxin precursor in _Zea_ seeds can be made. It seems likely however that the free IAA of the endosperm is not the source of the IAA in the shoot.

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