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Movement of Indole-3-acetic Acid and Tryptophan-derived Indole-3-acetic Acid from the Endosperm to the Shoot of *Zea mays* L.[1]

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ABSTRACT

The structures and the concentrations of all of the indolylic compounds that occur in the endosperm of the seeds of corn (*Zea mays* L.) are known. Thus, it should be possible to determine which, if any, of the indolylic compounds of the endosperm can be transported to the seedling in significant amounts and thus help identify the seed-auxin precursor of Cholodny (1935. Planta 23: 289–312) and Skoog (1937. J. Gen. Physiol. 20: 311–334). Of interest is the transport of tryptophan, indole-3-acetic acid (IAA), and the esters of IAA, which comprise 95% of the IAA compounds of the seed. We have shown that: (*a*) IAA can move from the endosperm to the shoot; (*b*) the rate of movement of IAA from endosperm to shoot is that of simple diffusion; (*c*) 98% of the transported IAA is converted into compounds other than IAA, or IAA esters, *en route*; (*d*) some of the IAA that has moved into the shoot has been esterified; (*e*) labeled tryptophan applied to the endosperm can be found as labeled IAA in the shoot; and (*f*) with certain assumptions concerning IAA turnover, the rate of movement of IAA and tryptophan-derived IAA from the endosperm to shoot is inadequate for shoot growth or to maintain IAA levels in the shoot.

We wish to determine which indolylic compounds of the seed serve as the source of indole-3-acetic acid (IAA) and IAA derivatives in the vegetative seedling. For *Zea* (see 5 for references) and *Avena* (13) the indolylic compounds of the seed are known, and for *Zea* it is known that the indolylic compounds occur mainly in the endosperm (14). It is not known which, if any, of these compounds move into the seedling to sustain growth.

Cholodny (2) discovered that a growth hormone diffused from *Avena* seeds when the seeds were put in water, or, to a lesser extent, in alcohol. Similarly, a piece of endosperm placed on a coleoptile produced and sustained curvature. Skoog (20) decapitated *Avena* coleoptiles and collected the seed auxin precursor in agar blocks placed on the seedling stumps. This diffused material induced curvature in the Went *Avena* test after 9 hr compared to 2 hr for free IAA. Van Overbeek (23), with others, showed that most of the IAA in the seed was a "bound" form and possibly served as the source of the IAA in the coleoptile tip.

More recently Hall and Medlow (9) identified IAA in phloem exudate and Whitehouse and Zalik (25) found that a radioactive substance with the R_F of IAA could be extracted from corn shoots after [^{14}C]IAA was injected into the endosperm. Sheldrake (19) found IAA and alkali-labile IAA compounds in the xylem sap of *Zea*. Auxin transport has been reviewed by Goldsmith (7) who observed (6) that upward movement of IAA in an anoxic seedling diffusion theory with a rate constant of 1×10^{-4} mm^2/sec. McCready (12) used inhibitors and low temperature to verify the existence of different mechanisms of upward and downward IAA transport.

In the present studies we show that IAA can move from endosperm to seedling, and that tryptophan applied to the endosperm appears as IAA in the seedling, but probably at rates that are too slow to provide the shoot with sufficient IAA. We have also shown that the transported IAA becomes esterified *en route*, and that 98% of the transported radioactivity is no longer in IAA or IAA conjugates. Thus, it is important that the method of measuring seedling IAA used in this work has been demonstrated to yield pure IAA (1). These results are a portion of the research done for a Master's dissertation (8).

MATERIALS AND METHODS

For determining total radioactivity, the tissues were combusted with a Packard model 306 Tri-Carb sample oxidizer. GLC was on a Varian 2740 gas chromatograph with a flame ionization detector and N_2 as the carrier gas. UV spectra were recorded with a Cary 15 spectrophotometer. Bray's solution (*cf.* 13) 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 were from the following sources: [2-^{14}C]IAA (23.5 mCi/mmol): Schwarz/Mann; [5-^{3}H]IAA (23.5 Ci/mmol): CEA France, obtained through M. H. Goldsmith of Yale University; DL-[5 (n)-^{3}H]tryptophan (25 Ci/mmol): Amersham/Searle; IAA and indole-3-butyric acid: Calbiochem; DEAE-cellulose; Dowex 50 W-X2 (H^+ form), 200–400 mesh: Sigma; Sephadex LH-20: Pharmacia; Silica Gel G plates: Merck Darmstadt-Brinkmann; 5% SP-2401 on Supelcoport: Supelco; bis(trimethylsily)tri-fluoroacetamide: Regis; Stowells Evergreen Hybrid Corn 1974 harvest: Ferry Morse Seed Co.

Plant Material. Corn kernels were surface-sterilized in 1% NaOCl for 10 to 20 min, soaked in aerated water for 16 hr, then placed in a row across a sterile paper towel. The towels were rolled, secured with tape, and placed in a beaker containing sterile water. When the beaker was full of towels it was covered with plastic wrap and incubated 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 cm long.

Application of Labeled Compounds. About one-third of the seed was cut off leaving the embryo, scutellum, and about 2 mm of the endosperm intact. All manipulations were at 25 C, 90% relative humidity, using a phototropically inactive green safelight and maintaining aseptic conditions by dipping the scalpel into alcohol. Five μl of radioactive compound in 50% ethanol was applied to the cut endosperm surface, corresponding to 7.2 ng and 2,100 dpm when [^{14}C]IAA was used, 7.6 ng and 2,264,800 dpm when [^{3}H]-IAA was used, and 24.8 ng and 6,745,600 dpm when [^{3}H]tryptophan 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 (about 5 mm from the point of

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emergence from the seed), and in some cases the roots (a few mm from the point of emergence), 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 membrane barriers other than those which any compound must permeate when moving from endosperm to shoot.

Tissue Combustion. Shoots and roots were combusted in groups of 10, the resultant CO_2 trapped in a quaternary amine base, or the ^3H trapped as ^3HOH, and the radioactivity counted in a liquid scintillation counter.

Reisolation of Free [^{3}H]IAA. The isolation procedure adopted requires almost 1 week for a single assay. Its sole advantage is that it has previously been shown to yield pure IAA from seedlings of *Zea* (1). 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% (v/v) acetone-water. The homogenate was extracted two more times with 70% acetone and the extracts combined and filtered. Five hundred μg each of IAA and 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 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 dissolved in 1 ml of $CHCl_3$. For the later experiments, $CHCl_3$ was substituted for ether extraction.

DEAE-cellulose column chromatography, Sephadex LH-20 chromatography, TLC, silylation, GLC, and UV spectrometry were carried out as previously described (1). The DEAE-cellulose column was prepared as described by Rouser *et al.* (17) for lipids, except that initially the column was washed with 100 ml of CH_3OH-CH_3COOH-$(C_2H_5)_3N$ (20:4:1), and then regenerated. The sample, in 1 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 $CHCl_3CH_3OH$-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-$CHCl_3$; (*d*) 300 ml of $CHCl_3$.

The sample from the DEAE-cellulose column was dissolved in 1 ml of 50% (v/v) ethanol-water and applied to a column of Sephadex LH-20 (22 × 1.8 cm) that had been washed with 50% ethanol. The sample was eluted with 50% ethanol at a flow rate of 3.5 to 4 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 μl of 50% ethanol and applied in a 10-cm streak across a thin layer Silica Gel G chromatography plate (20 × 20 cm). One-cm streaks of the sample and guide spots of IAA were applied on each side of the sample streak. 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 (4) to determine the migration of IAA in the sample. The region where IAA was present was scraped from the plate and the silica gel eluted three times with 5 ml of 50% ethanol-water sedimenting the silica gel by centrifugation for 10 min at 500*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 present, the sample was dissolved in 200 μl of 50% ethanol, transferred to a clean drying flask, and redried.

The sample was then dissolved in 200 μl of 50% ethanol, transferred to a serum vial, dried under N_2 at 70 C, and sealed with a serum cap. Silylation was with 10 μl of redistilled pyridine and 20 μl of bis(trimethylsilyl)-trifluoroacetamide added to the sealed vial with a syringe. The sample was reacted at 45 C for 15 min. GLC was on 5% SP-2401 on 100/120 mesh Supelcoport in a glass column (1.8 m × 6 mm) at 165 C, with 40 ml/min of N_2 as carrier gas. Fully silylated IAA was first injected into the column to determine its retention time; typically 15 min. A few μl of the sample were injected to determine the sample profile. The rest of the sample was injected, 7 μl at a time, and the silylated IAA collected by extinguishing the hydrogen flame and slipping a glass tube over the detector outlet during the emergence time of IAA. The silylated IAA, 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 to be certain the silylated IAA was spectrally pure. The 282 and the 225 nm peaks were used to determine the amount of IAA present. Any absorption by *p*-coumaric acid was corrected for by multiplying the sample at 330 nm by 4 or 3.3 and subtracting these values from the *A* at 282 and 225 nm, respectively. The molar extinction coefficient used for silylated IAA was 6060 at 282 nm and 33,200 at 225 nm (1), the same as for IAA.

One-half ml of the sample was added to 5 ml of Bray's solution and counted for 100 min in a liquid scintillation counter. A correction for the amount of radioactive IAA lost during purification was made by reverse isotope dilution. The percent recovery of [^{3}H]IAA should equal the per cent recovery of the 500 μg of unlabeled carrier IAA added at the beginning of the purification. Thus, the total radioactivity in the corn shoots was calculated from equation 1:

$$\frac{\text{Carrier IAA added}}{\text{Carrier IAA recovered}} \times (\text{radioactivity of recovered sample}) = \text{radioactivity in shoots} \quad (1)$$

Reisolation of Free Plus Alkali-labile [^{3}H]IAA. 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 1 hr 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 additonal determination of the amount of IAA recovered was made with the Ehmann assay (4, 13). Estimates of the amount of IAA recovered based on A_{282}, A_{225} and the Ehmann color test agreed closely.

Determination of Tryptophan in Seeds. The shoots and roots were removed from 4-day-old corn seedlings and the seeds extracted with 70% acetone-water and filtered. Radioactive tryptophan was added to the extract, the extract evaporated to dryness, and the residue dissolved in 5 ml of water. After adjusting the pH to 2.5 the sample was extracted three times with 15 ml of chloroform. The water portion was evaporated to dryness, taken up in 1 ml of water and applied to a Dowex 50-H^+ column. The column was washed with 3 bed volumes of water, then with 2 N NH_4OH, and the tryptophan collected at 1½ bed volumes of NH_4OH. The tryptophan-containing fractions were combined, evaporated to dryness, dissolved in 1 ml of 50% ethanol-water (v/v), and applied to a column of Sephadex LH-20 (2.2 × 1.8 cm) previously equilibrated with 50% ethanol. The sample was eluted with 50% ethanol between 27 and 30 ml. Fractions containing tryptophan were evaporated to dryness, dissolved in 200 μl of 50% ethanol, and applied to a TLC plate. The solvent used to develop the plate was: ethyl acetate-methyl ethyl ketone-ethyl alcohol-water (5:3:1:1). The gel containing tryptophan was scraped from the plate and eluted three times with 50% ethanol. A colorimetric assay (4, 13) was used to determine the amount of tryptophan recovered and a liquid scintillation counter was used to measure the radioactivity

of the sample. Knowing the amount of labeled tryptophan added to the acetone extract (X), its specific activity (C_0) and the specific activity of the isolated tryptophan (C), the amount of tryptophan (y) in the seeds was calculated from equation 2 (16):

$$y = \left(\frac{C_0}{C} - 1\right)X \qquad (2)$$

RESULTS

Movement of Radioactivity from Endosperm to Shoot and Root. The CO_2 derived from combustion of the shoots or roots contained radioactivity within 1 hr after 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 10 roots. After 8 hr, about 30 cpm were found in each shoot and with a counting efficiency of 77%; this represents 1.9% of the radioactivity applied. If all of the radioactivity were still in IAA, and there was no isotope dilution, this would correspond to 1.3×10^{-10} g of IAA/shoot.

The amount of [^{3}H]OH obtained by combustion of the shoots after application of [^{3}H]tryptophan to the seeds can be seen in Figure 2. Each point is the average of two groups of 10 shoots. After 8 hr 10,000 cpm were found in the shoot. At a counting efficiency of 33% this represents 0.5% of the applied radioactivity and assuming no isotope dilution, would correspond to 1.1×10^{-10} g of tryptophan/shoot.

IAA in the Shoot Derived from [^{3}H]IAA Applied to the Seed. The results of experiments in which [^{3}H]IAA was reisolated from the shoot after an 8 hr incubation time are shown in Table I. The radioactivity data were corrected for recovery of the 500 μg of IAA added to the acetone extract and for the 33% counting efficiency for ^{3}H. The average amount of IAA that moved from the endosperm was 1.6×10^{-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 samples hydrolyzed after concentration of the acetone extract. After correcting for equilibration of the [^{3}H]IAA with the endogenous pool of free IAA in the endosperm (730 μg/kg; unpublished data of J. Cohen in this laboratory) a value of 2.6×10^{-11} g of IAA transported per shoot is obtained. If the applied [^{3}H]IAA also equilibrated with the endogenous bound IAA which Ueda and Bandurski (21) found to be 6 mg/kg in 4-day-old seedlings the amount of IAA moved to the shoot would be 2.2×10^{-10} g/shoot. This is, however, unlikely since Kopcewicz *et al.* (11) have shown that the ester pool is only slowly labeled 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 (21) showed that 1% of the esterified IAA is lost each hr for the first 96 hr of germination and the present experiment involved 8 hr between application of [^{3}H]IAA and extraction. This assumes that the pathway for ester 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 4×10^{-11} g/shoot.

These calculations were made using the average radioactivity found in a shoot for the hydrolized samples, 466 dpm. It was assumed that two-thirds of the seed remained after cutting and that 417 seeds weighed 100 g.

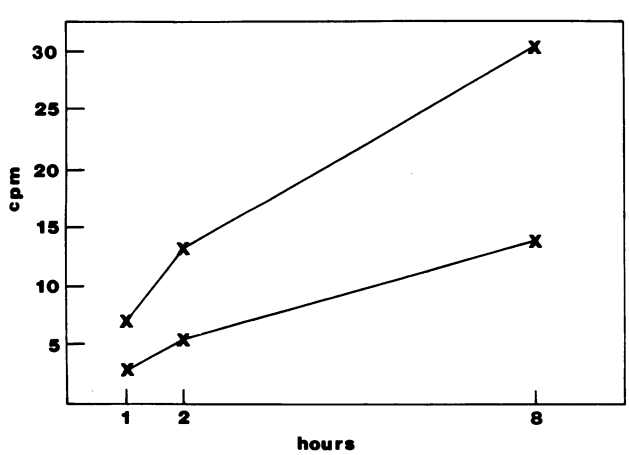


FIG. 1. Radioactivity found in the shoot (upper curve) and root (lower curve) of a corn seedling after 2100 dpm of [^{14}C]IAA were applied to the endosperm.

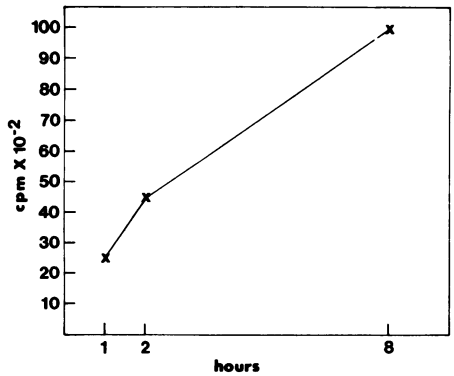


FIG. 2. Radioactivity found in the shoot of a corn seedling after application of 2.2×10^6 dpm of [^{3}H]tryptophan to the endosperm.

TABLE I

Amount of ^{3}H-IAA isolated from corn shoots after application of ^{3}H-IAA to the endosperm

Experiment	Treatment	Recovery of IAA by: A_{280}	Recovery of IAA by: Ehmann Assay	Radioactivity in: ½ the sample	Radioactivity in: 60 shoots	IAA per shoot
		%	%	cpm	dpm	g × 10^{13}
1	not hydrolyzed	1.5	1.9	25	10.000	5.6
"	hydrolyzed	2.4	2.3	68	17.000	9.5
2	not hydrolyzed	4.0	3.1	104	15.600	8.7
"	hydrolyzed	4.9	4.0	165	20.204	11
3	not hydrolyzed	3.6	2.8	78	13.000	7.3
"	hydrolyzed	1.4	0.9	109	46.714	26

TABLE II

Amount of ^{3}H-IAA isolated from corn shoots after application of ^{3}H-Tryptophan to the endosperm

Experiment	Treatment	Recovery of IAA by: A_{280}	Recovery of IAA by: Ehmann assay	Radioactivity in: ½ the sample	Radioactivity in: 60 shoots	IAA per shoot
		%	%	cpm	dpm	g × 10^{13}
1	hydrolyzed	2.8	2.3	25	5357	2.8
2	hydrolyzed	3.3	2.6	17	3091	1.6

IAA in the Shoot Derived from [^{3}H]Tryptophan Applied to the Seed. When [^{3}H]tryptophan was applied to the corn endosperm, [^{3}H]IAA was recovered from the shoot. An average of 2.2×10^{-13} g of [^{3}H]IAA derived from [^{3}H]tryptophan was found in the shoot as seen in Table II. When corrections are made for dilution of the applied [^{3}H]tryptophan with the tryptophan pool in the seed (33.3 mg/kg wt), the amount of IAA derived from tryptophan found in the shoot is calculated to be 6×10^{-11} g of IAA, and 3.5×10^{-11} for the two experiments for an average of 4.8×10^{-11} g of IAA.

DISCUSSION

Six and possibly seven, conclusions can be drawn from this work. First, IAA applied to corn endosperm can be reisolated from the shoot (Table I). This confirms earlier work (25) but is the first time that this has been demonstrated using an isolation procedure that is known to yield pure IAA. Second, 98% of the radioactivity of the labeled IAA applied to the endosperm is no longer IAA, or IAA conjugates, by the time it has reached the shoot (Table I and Fig. 1). Third, labeled IAA becomes esterified *en route* either in the endosperm or in the shoot (Table I). 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 and this corresponds to the rate of simple diffusion (see below). Fifth, a diffusion constant can be calculated for radioactivity of tryptophan moving from endosperm to the seedling (see below). Sixth, tryptophan applied to the endosperm is slowly converted to IAA found in the seedling (Table II). Last, if certain assumptions are made concerning the rate of turnover of IAA and tryptophan, that is, rate of formation and destruction of these compounds, a tentative conclusion concerning the amount of IAA transported and the amount of IAA in the shoot derived from endosperm tryptophan can be made (Table III).

Diffusion Constants for IAA. Assuming the 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 calculated with equation 3:

$$C/C_0 = 1 - \frac{2}{\sqrt{\pi}} \int_0^{\frac{X}{2\sqrt{Dt}}} e^{\frac{-x^2}{4Dt}}\, dx = \operatorname{erfc} \frac{X}{2\sqrt{Dt}} \qquad (3)$$

where C_0 is the concentration at the source, C is the concentration in the section, and X is the distance between the source and the section (6). In calculating the diffusion constant we assumed that the radioactivity applied spread out into a two-dimensional, volumeless plane, and that the radioactivity in the shoot was also in a two-dimensional volumeless plane at the surface where the shoot was cut. This permits setting C/C_0 equal to 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 previously published tables (3). When:

$$\frac{C}{C_0} = \frac{31}{2100} = .01428 \qquad (4a)$$

$$\frac{x}{2\sqrt{Dt}} = 1.7 \text{ (from table)} \qquad (4b)$$

$$D = 3 \times 10^{-6}\ \text{cm}^2/\text{sec} \qquad (4c)$$

Thus, the diffusion constant for radioactivity moving 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. Then:

$$\frac{C}{C_0} = \frac{466}{2{,}210{,}500} = .000211 \qquad (5a)$$

$$\frac{X}{2\sqrt{Dt}} = 2.6 \qquad (5b)$$

$$D = 1.3 \times 10^{-6}\ \text{cm}^2/\text{sec} \qquad (5c)$$

The diffusion constant for [^{3}H]IAA is 1.3×10^{-4} mm^2/sec. This value for [^{3}H]IAA is comparable to the diffusion constant of 1.26×10^{-4} mm^2/sec found by Goldsmith (6) for basal movement of IAA by anoxic oat coleoptiles and using the same equations and assumptions.

A diffusion constant can also be calculated for the movement of radioactivity to the shoot when [^{3}H]tryptophan was applied to the endosperm. In this case:

$$\frac{C}{C_0} = \frac{3 \times 10^4}{6.6 \times 10^7} = .000454 \qquad (6a)$$

$$\frac{x}{2\sqrt{Dt}} = 2.5 \qquad (6b)$$

$$D = 1.4 \times 10^{-6}\ \text{cm}^2/\text{sec} \qquad (6c)$$

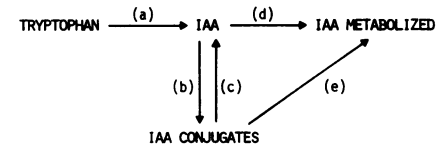
Thus, the diffusion constant for [^{3}H]IAA derived from [^{3}H]tryptophan applied to the endosperm is 1.4×10^{-4} mm^2/sec.

Pollack (15) found that the principal barrier to diffusion of ^{133}Xe through frog skins and toad bladders was inter- and intracellular water. By using the diffusion constant for Xe through water divided by the thickness of frog skin or toad bladder he calculated rates of 2.4×10^{-3} mm/sec and 12×10^{-3} mm/sec, respectively. These values are close to measured diffusion constants of 3.9×10^{-3} mm/sec and 7.4×10^{-3} mm/sec for frog skin and toad bladder. The principal barrier to IAA diffusion may also be water and IAA would diffuse in a manner similar to Xe except that the negative charge on IAA and its higher mol wt would make IAA diffusion slower than that of Xe. The diffusion constant of Xe through water is 12×10^{-4} mm^2/sec (22). This value is larger than the diffusion constant we find for IAA. Thus, simple diffusion can account for the movement of IAA from the seed to the shoot.

Physiological Significance of the Transport of IAA and of Tryptophan-derived IAA from the Endosperm to the Seedling.

There are insufficient data to estimate the rate at which IAA is made, and destroyed, that is the "IAA-flux" in the endosperm. Without this knowledge, the specific activity of the IAA in the endosperm, as it enters the seedling, is not known, and radioactivity of IAA cannot be converted into amount of IAA. Nonetheless, there are sufficient data on amounts of IAA and IAA conjugates in the endosperm and the rate of disappearance of these conjugates during germination to make assumptions concerning the flux of IAA in the endosperm. There are also data concerning the amounts of IAA required in the seedling (1, 24) and so a tentative assessment of the adequacy of this transport is possible.

The flux of IAA in the endosperm can be represented by the following, previously diagrammed (15), scheme:



The conversion of tryptophan to IAA (a) is a known reaction (*cf.* 18 and this communication). The formation of IAA conjugates from IAA (b) has been studied in *Zea, in vitro* (11) and *in vivo* (this communication). The release of free IAA from IAA esters (c) has been shown, indirectly, for *Zea* seedlings (10). Whether IAA conjugates can be metabolized directly (e) without going through free IAA, is not known. We assume that (e) does not occur, that (d) does not affect our conclusions since IAA concentrations remain steady-state (21) and [^{14}C]IAA equilibrates with unlabeled IAA and we show in this communication that (a) is too slow to affect our conclusion. The rate at which IAA is being made is the rate of reaction (c) and this has been shown to be 1% of the ester content per hr during the first 96 hr of germination (21). If reaction (c) did not occur then the isotopic IAA applied to the endosperm would be diluted only by the endogenous IAA. Using this specific radioactivity, the amount of IAA transported would be 2.6×10^{-11} g/shoot. If the labeled IAA is diluted by the

TABLE III

IAA transport from endosperm to shoot

Basis for calculation	Amount of IAA transported into shoot
Assuming no dilution of IAA applied to endosperm	1.6×10^{-12} g/shoot
Assuming dilution with free IAA in the endosperm	2.6×10^{-11} g/shoot
Assuming dilution with free IAA plus 8% of the IAA esters in the endosperm	4×10^{-11} g/shoot
Assuming no dilution of tryptophan applied to endosperm	2.2×10^{-13} g/shoot
Assuming dilution with tryptophan in the seed	4.8×10^{-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^{-4}M IAA in the shoot	1.3×10^{-8} g/shoot

endogenous free IAA, plus 8% (1% per hr × 8 hr) of the ester IAA, then the amount of IAA transported would be 4×10^{-11} g of IAA per shoot. These values are tabulated in Table III.

Similar calculations can be made for tryptophan if we assume that the labeled tryptophan is only diluted by the free tryptophan of the endosperm. A value of 4.8×10^{-11} g of IAA per shoot is obtained. This value is also given in Table III.

It is possible to estimate how much IAA is diffusing from the tip down and thus how much is supplied by the endosperm. Went and Thimann (24) showed that maximal curvature of oat coleoptiles occurs with 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 the block enters the plant. This corresponds to 3×10^{-10} g of IAA per plant for optimal curvature. If this auxin is originating in the seed, the seed must be supplying 3×10^{-10} g of IAA to the seedling. This value is also in Table III.

Another estimate of how much IAA moves from endosperm to shoot is possible since it is known that the concentration of IAA and IAA esters in shoots of *Zea* is 2×10^{-6} M (1). The average growth in a shoot during the 8 hr period was 0.036 g/shoot. The growth is predominantly an increase in water. IAA, from the seed, would presumably keep the concentration of IAA in the shoot at a constant level. Using these data, we conclude that the amount of IAA which must move from endosperm to shoot in an 8-hr period, to maintain a tissue concentration of 2×10^{-6} M, would be 1.3×10^{-8} g. Thus, in a 6- to 8-hr period, the endosperm must supply the shoot with between 3×10^{-10} g to 1.3×10^{-8} g of IAA. The lesser amount would, presumably, be just physiologically adequate while the higher amount would provide sufficient IAA to maintain the known ester pool (1). Our best estimate of the amount of IAA that moves from endosperm to shoot during 8 hr is 4×10^{-11} g and this would be between 0.3% to 13% of the estimated necessary amount. When labeled tryptophan is applied to the endosperm, about 4.8×10^{-11} g of labeled IAA appears in the shoot and this would provide between 0.4 to 16% of the necessary amount. Since the amounts of IAA supplied to the vegetative tissue by endosperm-applied IAA or tryptophan range from about 1 to 10% of that deemed adequate, we conclude that these calculations raise doubts as to whether the movement from endosperm to shoot of IAA and tryptophan-derived IAA is sufficient to provide the physiological needs of the young vegetative tissue.

While 1.9% of the applied radioactivity was recovered from shoots with complete oxidation of the tissue, only 0.02% of the applied radioactivity could be reextracted as IAA. Thus, metabolism of the radioactive IAA transported to things other than IAA or IAA esters accounts for 98% of the radioactivity that moved to the shoot.

Further studies with labeled IAA esters are required before a decision as to the identity of the diffusible auxin precursor in *Zea* seeds can be made. At present it seems unlikely that free IAA, or free tryptophan of the endosperm is the source of the IAA or IAA esters in the shoot.

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