Plant Physiol. (1980) 65, 415–421
0032-0889/80/65/0415/07/$00.50/0

Concentration and Metabolic Turnover of Indoles in Germinating Kernels of *Zea mays* L.[1]

Received for publication May 15, 1979 and in revised form September 19, 1979

EPHRAIM EPSTEIN,[2] JERRY D. COHEN, AND ROBERT S. BANDURSKI[3]
Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

The amounts and rates of metabolic turnover of the indolylic compounds in germinating kernels of sweet corn were determined. Knowledge of pool size and rate of pool turnover has permitted: (*a*) identification of indole-3-acetyl-*myo*-inositol as the major chemical form for transport of indole-3-acetic acid (IAA) from endosperm to shoot; (*b*) demonstration that the free IAA of the endosperm is turning over rapidly with a half-life of 3.2 hours; (*c*) identification of esters of IAA as the immediate precursors of IAA in the endosperm and shoot; (*d*) demonstration that neither tryptophan nor tryptamine is a major precursor of IAA for the seed or shoot; (*e*) identification of IAA-*myo*-inositol glycosides as precursors of IAA-*myo*-inositol.

It is concluded that seedlings of *Zea mays* utilize esters of IAA, and not tryptophan or its derivatives, for the IAA requirements of the germinating seedling.

The endosperm of kernels of *Zea mays* contains small amounts of indole-3-acetic acid and large amounts of esterified IAA (5, 15, 26, 27, 35, 38). The esterified IAA has been chemically characterized (1, 21, 26, 36) and assayed quantitatively in the dry seed (35) and during ripening (9 and unpublished data) and during germination of the kernels (ref. 35 and unpublished data). It remained necessary to measure the rate of metabolic turnover of the indolylic compounds to permit determining which are being exported from the kernel to the shoot and to search for metabolic functions.

We previously demonstrated that: (*a*) the esters play a role in hormonal homeostasis in the seedling vegetative shoot (4); and (*b*) that esterification plays a protective role in preventing peroxidative attack upon IAA (8). Now, with knowledge of turnover in the endosperm, we can add additional functions for the esters; they are: (*c*) transport of IAA-*myo*-inositol from the kernel to the shoot in amounts sufficient to provide the seedlings needs (24); and (*d*) providing the kernel with a large and renewable amount of free IAA during germination. The resultant free IAA is, in small part, transported to the shoot, with the bulk decarboxylated or otherwise metabolized in the endosperm. The rates of destruction and formation of IAA are equal so that the amount of free IAA in the endosperm remains steady-state. Neither tryptophan nor tryptamine serves as a major source of IAA in *Zea* kernels.

We also demonstrate that the IAA-*myo*-inositol esters turnover at a rate too great to be accounted for by transport into the shoot or deacylation or irreversible hydrolysis of the glycosidic moiety of IAA-*myo*-inositol glycosides (36). A glycosylation function for IAA-*myo*-inositol arabinose and IAA-*myo*-inositol galactose (1) is suggested.

Prior studies (28, 30, 31, 34) have dealt with the conversion of tryptophan, tryptamine, and indole-3-ethanol to IAA; to our knowledge this is the first study of both the amounts and the kinetics of turnover of tryptophan, IAA, and IAA esters in plant tissue. Inasmuch as this is an initial attempt, imperfect knowledge of possible pool multiplicity and short term kinetic perturbations cause us to believe that only "order of magnitude" conclusions are, as yet, possible.

MATERIALS AND METHODS

Plant Tissue. Corn kernels (*Z. mays*, var. Stowell's Evergreen Sweet Corn) were surface-sterilized in 1% NaOCl for 10–20 min, soaked in running water at 25 C for 16 h, then placed in rows across paper towels. The towels were rolled, secured with tape, placed in a beaker containing water and incubated in the dark for an additional 80 h. About 30% of the endosperm was cut from the end of the kernels leaving the embryo and scutellum intact, so that an endosperm surface was exposed for isotope application. All manipulations were at 25 C, 90% RH, and with use of a phototropically inactive green safelight.

Application of Labeled Compounds—Recovery Corrections. Ten μl of the radioactive compound in glass-distilled H_2O was applied to the cut endosperm surface. Each 10-μl aliquot contained 0.6 ng and 209,000 dpm in the case of L-[5(n)-^{3}H]tryptophan (27.5 Ci/mmol, Amersham), or 1.6 ng and 1,233 dpm [2-^{14}C]IAA (57.2 mCi/mmol, New England Nuclear) or 28 ng containing 20,317 dpm (57.2 mCi/mmol, Amersham) [1-^{14}C]IAA or 12.9 ng and 730 dpm [2-^{14}C]indole-3-acetyl-*myo*-inositol (4.5 mCi/mmol), a gift from Dr. Janusz Nowacki (25). The amount of isotopically labeled compound applied was 0.003, 3.0, and 1.0% of that already present in the endosperm for tryptophan, IAA, and IAA-*myo*-inositol, respectively, so perturbation of metabolic pools was minimized. For zero time determinations of pool size, the kernels, following removal of shoot and root, were immediately dropped into acetone in Waring Blendor jars at −70 C. For experiments measuring turnover, the kernels were pinned to styrofoam sheets and incubated for 4–8 h in a moist chamber. Shoots and roots were removed and the kernels dropped into acetone at −70 C as above. The amount of acetone was adjusted so that the final acetone concentration, after allowance for water in the kernels, was 70% (v/v).

Extraction of Free IAA. The kernels (800–1,000) were extracted by grinding in 70% acetone for 2 min in a 4 liter (CB-6) Waring Blendor. Ten ml of acetone, containing 1 mg indole-3-butyric acid, as carrier for the IAA, was added to each homogenate. The homogenate was kept at 4 C for 16 h, then filtered and the residue resuspended in 70% acetone twice for 12 h. The filtrates were combined and the volume reduced to 100 ml on a flash evaporator

[1] This work was supported by grants from the Metabolic Biology Section of the National Science Foundation, PCM 76-12356. Journal Article 8953 from the Michigan Agricultural Experiment Station.

[2] Accomplished this research while on sabbatical leave from the Department of Horticulture, The Volcani Center, Bet Dagan, Israel.

[3] To whom reprint requests should be addressed.

(12 mm Hg, bath temperature, 50 C). The concentrate (pH 4.0) was adjusted to pH 2.5 with 12 N H_2SO_4 and extracted three times with 200 ml of $CHCl_3$. The combined chloroform phases were dried over anhydrous granular Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was dissolved in 4 ml $CHCl_3$ for column chromatography.

Isolation and Quantitative Estimation of IAA. Chromatography on DEAE-cellulose, Sephadex LH-20, and TLC were as described (2, 3). The amount of IAA isolated was determined by the Ehmann assay (12, 24) and radioactivity by scintillation counting in ACS solution (Amersham). Then the amount of IAA in the tissue was determined by the isotope dilution equation (29) discussed below.

Assay of Conversion of [^{3}H]Tryptophan to [^{3}H]IAA. The procedure of extraction described above was employed except that [^{3}H]tryptophan was applied to the endosperm and any [^{3}H]IAA formed was isolated from the endosperm following the addition of carrier IAA as described by Hall and Bandurski (17).

Extraction and Quantitative Estimation of Tryptophan in Kernels. The water remaining after $CHCl_3$ extraction of acidic compounds was evaporated *in vacuo* to 50 ml and applied to a Dowex 50 W-X2 H^+ form (200–400 mesh) column (1 × 25 cm) using a flow rate of 0.3 ml min^{-1} (13). The column was washed with 150 ml water and eluted with aqueous 2 M NH_4OH. Tryptophan was eluted between 10 and 30 ml as monitored by its radioactivity. The tryptophan-containing tubes were combined and evaporated to dryness; the residue was dissolved in 1 ml 50% 2-propanol, applied to Sephadex LH-20 column (1.8 × 18 cm), and eluted with 50% 2-propanol using a flow rate of 0.3 ml min^{-1}. Tryptophan was eluted between 30 and 60 ml. The tryptophan-containing tubes were pooled, reduced in volume to 0.5 ml, and chromatographed on Whatman 3MM paper using water as solvent. Tryptophan was extracted from the paper with water and rechromatographed on silica gel 60 plates (E. Merck, Darmstat-Brinkmann) with methyl ethyl ketone-ethyl acetate-absolute ethanol-water (3:5:1:1) as solvent (21). Tryptophan was eluted from the silica gel with 3 ml 50% 2-propanol. The specific activity of [^{3}H]tryptophan was determined by means of the Ehmann reagent (12, 24) and by scintillation counting. No significant tritium exchange occurred during the above described isolation procedure.

Quantitative Estimation of Tryptophan in Shoots. Four-day-old corn shoots (84 g) were ground in 80% acetone; 1 μCi methylene-[^{14}C]tryptophan (57 mCi/mmol, Amersham) was added, and the insoluble residue removed by filtration. The filtrate was adjusted to pH 2.5, extracted with chloroform, and the resultant water phase was reduced in volume to 3 ml. Following chromatography on a column (1 × 50 cm) of Sephadex LH-20 with 50% 2-propanol, the tryptophan-containing tubes were pooled, reduced in volume, and chromatographed on a Dowex 50 W-X2 H^+ form column as above. The tryptophan-containing tubes were pooled, reduced in volume, and then column chromatographed on silica gel 60 with elution with methyl ethyl ketone-ethyl acetate-absolute ethanol-water (3:5:1:1) followed by 50% 2-propanol. The sample was applied to a silica gel 60 thin layer plate and developed as described above for tryptophan. The specific radioactivity of the [^{14}C]tryptophan was determined by means of the Ehmann reagent (12, 24) and by scintillation counting.

Extraction and Quantitative Estimation of Tryptamine. To determine the amount of tryptamine, 4.76 μg [G-^{3}H]tryptamine (41,158,000 dpm, 1.65 Ci/mmol, Amersham) were added to a 70% acetone homogenate of whole kernels (1.9 kg) and the tryptamine reisolated for measurement of isotope dilution. The extract was reduced in volume to 100 ml and adjusted to pH 11.0 with solid NaOH. The solution was extracted three times with equal volumes of $CHCl_3$, and the pooled $CHCl_3$ extracts evaporated to dryness. The residue was taken up in the minimal volume of 0.1 M Na-citrate-acetic acid buffer (pH 4.0) in 50% ethanol and put on a Dowex 50 W-X2 H^+ form (200–400 mesh) column (1 × 25 cm) in 0.2 M citrate buffer (pH 4.0). The column was washed with 100 ml of the same buffer followed by 100 ml of 50% ethanol.

Tryptamine was eluted with a 50% aqueous ethanol-ammonia gradient. Tryptamine was eluted when the buffer contained between 0.4–0.45 N ammonia. Tubes containing tryptamine (45–155 ml) were pooled and evaporated to 1 ml. A sample was chromatographed on a silica gel plate with 2-propanol-acetic acid-water (90:5:5) as solvent. The tryptamine-containing band was scraped off and eluted with 5 ml 50% 2-propanol. [^{3}H]Tryptamine was counted and the amount measured by the Ehmann reagent (12, 24) and thus, its specific radioactivity determined.

Extraction and Quantitative Estimation of IAA-*myo*-Inositol. Ten μl of [^{14}C]indole-acetyl-*myo*-inositol were applied to the cut endosperm surface of 500 kernels, as described above. At zero time, or after 8 h of incubation, the kernels were ground, extracted three times with 200 ml 70% acetone, the extracts were filtered, reduced in volume *in vacuo* to 40 ml, and stored 18 h at 4 C. The solution was filtered through Whatman No. 1 paper, then through No. 42 paper and reduced in volume to 3 ml. The syrupy solution plus 2 ml of water wash were applied to a Dowex 50 W-X2 H^+ form (200–400 mesh) column (30 × 1 cm) (13). Prior to use, the column was washed with 200 ml of 100 mM Na-citrate (pH 5.6) followed by 0.5 liter distilled H_2O. The column was eluted with 1 mM Na-citrate (pH 6.2), with a flow rate of 4 ml h^{-1} and collecting 3 ml per tube. The IAA-inositol esters were eluted between 60 and 200 ml. This fraction was evaporated to dryness, dissolved in 0.5 ml 50% 2-propanol, and applied to a column (17 × 0.9 cm) of Beckman PA 28 resin for high pressure liquid chromatography (7). The column was eluted with 50% 2-propanol at 8.2 atm pressure with a flow rate of 0.25 ml min^{-1} and collecting 1 ml $tube^{-1}$. Under these conditions IAA-inositols were eluted between 18 and 80 ml. The IAA-inositol-containing fractions were pooled, reduced in volume, and applied to silica gel 60 plates. Methyl ethyl ketone-ethyl acetate-absolute ethanol-water (3:5:1:1) was used as solvent (21) and the IAA-inositols visualized on a guide strip with the Ehmann reagent (12, 24). The unsprayed band in the R_F region of the IAA-*myo*-inositols (13) was eluted with 5 ml 50% 2-propanol. After reduction in volume, the specific radioactivity was determined using the Ehmann reagent (12, 24) and scintillation counting.

Decarboxylation of [1-^{14}C]IAA. To determine the rate at which IAA was decarboxylated by corn endosperm, 10 μl [1-^{14}C]IAA containing 28 ng and 20,750 dpm was applied to the cut endosperm surface of corn seedlings. Two such seedlings were inserted into each of eight vials (14) together with a small vial containing 1 N NaOH. The vials were stoppered and incubated at 25 C in darkness for 8 h. The NaOH was transferred to scintillation vials and the radioactivity measured.

Determination of Concentration of IAA in Kernels as a Function of Germination Time. The purification procedure used was slightly modified from that previously described (17). Dry, ungerminated kernels (500 g) were first ground in a hammer mill then treated as the germinated kernels. For germinated kernels (800–1,000 kernels), the emerging shoot and root tissue was first removed and the kernels ground in a Waring Blendor in 50% acetone. The tissue was extracted three times with 600 ml 50% acetone-water during a 24-h period. The combined extracts were filtered, pooled, reduced in volume to 150 ml, and chromatographed on Dowex 50 as described for ester purification. Fractions containing IAA were pooled, acidified to pH 2.5, and extracted three times with $CHCl_3$. After removal of the $CHCl_3$, the residue was suspended in 100 μl 50% 2-propanol and chromatographed on a silica gel plate using chloroform-methanol-water (85:14:1). The area containing IAA was eluted and assayed with Ehmann's reagent and radioactivity determined by scintillation counting.

RESULTS

RATIONALE FOR EXPERIMENTS

The isotope dilution technique was used to measure the amounts of each indolylic compound present in the kernel at zero time.

Continued dilution of the applied labeled compound, as a function of incubation time, was then used to estimate turnover. Once pool size and turnover were known it became possible to translate radioactivity transported from endosperm to shoot into amounts of compound transported from endosperm to shoot. A theoretical and experimental basis for such calculation has been detailed earlier (6, 23, 32).

As in the accompanying paper (24) the following data and approximations are used:

a. There is an average of 4,348 kernels kg^{-1} dry weight of kernels so that each kernel weighs 0.23 g.

b. Excision of a portion of the endosperm to permit isotope application removes 31% of the endosperm. Thus, cut seed data are multiplied by 1.45 to convert to whole plant data.

c. The experiments were of 8-h duration. Since specific radioactivities of the compound applied were determined at 0 and 8 h, the specific radioactivity at 4 h was either measured or calculated to be used as the "average" specific radioactivity of the compound as it moved out of the endosperm and into the scuteller absorbing surface. Since dilution of specific radioactivity is a first order reaction, the mean specific radioactivities would have occurred at 3.3, 3.6, and 3.8 h for IAA, tryptophan and IAA-*myo*-inositol, respectively. However, we deal with orders of magnitude, so correction to the arithmetic mean was adequate for our purposes.

d. The pool size of total IAA ester, A + B fraction, after 4 days of germination would be about 27,000 pmol $kernel^{-1}$ and the rate of ester "disappearance" is known to be 1% h^{-1} (35).

e. As determined in this paper, the pool size of free IAA was 308 pmol $kernel^{-1}$ and the specific radioactivity of IAA 4 h after application to the endosperm was calculated to be 43% of that at zero time.

f. As determined in this paper, the pool size of tryptophan at zero time is 87,000 pmol $kernel^{-1}$ and at $t = 4$ h the specific radioactivity was calculated to be 58% of that at zero time.

g. As determined in this paper, the pool size of IAA-*myo*-inositol at zero time is 6800 pmol $kernel^{-1}$ and at $t = 4$ h the specific radioactivity was calculated to be 80% of that at zero time.

CALCULATIONS

The amount of IAA, IAA-inositol, tryptophan, or tryptamine at zero time was determined by isotope dilution analysis of kernels immersed in dry ice-acetone and ground immediately after application of one of the above labeled compounds. Then the amount of the compound in the kernels, Y, was:

$$Y = \left(\frac{C_i}{C_o} - 1\right) X$$

where C_i was the initial specific radioactivity of the labeled compound, C_o its specific radioactivity upon reisolation at zero time, and X, the amount of labeled compound applied (3, 29).

In experiments measuring metabolic turnover, the decrease in C_o, as a function of time, was measured. Following incubation the shoots and roots were removed and the kernels dropped into dry ice-acetone as for the zero time samples. About 800–1,000 kernels were used for each measurement and the specific radioactivity of the applied compound was determined 4 or 8 h after application of the labeled compound. Any compound being made would show decreased specific radioactivity as a function of time of incubation. The first order rate constant, k, was then calculated from the equation (6, 32, 39):

$$\log \frac{C_o}{C_t} = \frac{kt}{2.303}$$

where C_o was the specific radioactivity of the labeled compound when reisolated at zero time after application to the endosperm; C_t, the specific radioactivity at time t; k, the first order rate constant; and t, time in h. The half-time, or turnover time ($t_{1/2}$) for a 50% reduction in specific radioactivity (the time required for the seed to make an amount of the constituent equal to the amount present initially) could then be calculated from the relationship:

$$t_{1/2} = \frac{\ln 2}{k}$$

The assumptions involved in calculating k and $t_{1/2}$ are discussed elsewhere (6, 32, 39; and see under "Discussion"). The purpose of this work was to determine whether turnover did or did not occur, and at approximately what rate. For these answers it is immaterial whether the reduction in specific radioactivity was zero, first, or, even second order since the estimation of turnover time would not change by more than 10–25%. Our data showed apparent first order kinetics so, when necessary, C_{4h}, the arithmetic mean point of our experiments, was calculated using k as a first order rate constant.

FORMATION OF IAA IN ENDOSPERM

The amount of free IAA, as calculated from the initial specific radioactivity, and the rate of change of specific radioactivity as a function of time after application of the isotope to the endosperm are shown in Table I. As can be seen the specific radioactivity

Table I. *Concentration and Metabolic Turnover of Some Indolylic Compounds in Zea Kernels*

Compound	Incubation Time	Recovered Specific Radioactivity	k	$t_{1/2}$
	h	*dpm/μg*	h^{-1}	*h*
	0	31,000		
IAA[a]	4	8,900	0.22	3.2
	8	5,400		
Tryptophan[b]	0	15,200		
	8	5,060	0.14	5.0
IAA-*myo*-inositol[c]	0	935		
	8	590	0.06	12.0

[a] 1.6×10^{-3} μg of 7.26×10^5 dpm/μg IAA was applied to each cut kernel. The average specific radioactivity of the IAA upon reisolation at $t = 0$ was found to be 3.2×10^4 dpm/μg so the amount of IAA in each intact kernel was:

$$Y = \left(\frac{7.26 \times 10^5}{3.12 \times 10^4} - 1\right)(1.6 \times 10^{-3})(1.45) = 5.2 \times 10^{-2}\ \mu g/kernel$$

Under identical conditions, the specific radioactivity at 4 and 8 h was 8.9×10^3 dpm/μg and 5.4×10^3 dpm/μg. Thus,

$$\log \frac{3.1 \times 10^4}{5.4 \times 10^3} = \frac{kt}{2.3} \text{ so that } k = 0.22 \text{ and } t_{1/2} = \frac{\ln 2}{0.22} = 3.2 \text{ h}$$

[b] A total of 0.6 μg tryptophan (1.79×10^5 dpm) was applied to each endosperm and the specific radioactivities upon reisolation were as shown.

[c] A total of 6.44 μg [^{14}C]IAA-*myo*-inositol containing 6.07×10^4 dpm/μg IAA were applied to a batch of 500 kernels. Upon reisolation at $t = 0$ the average specific radioactivity was found to be 9.35×10^2 dpm/μg. Thus,

$$Y = \frac{\left(\frac{6.07 \times 10^4}{9.35 \times 10^2} - 1\right)(6.44)}{500} \times 1.45 = 1.19\ \mu g/\text{intact kernel}$$

$$= 6{,}800 \text{ pmol kernel}^{-1}$$

Under identical conditions, the specific radioactivity at $t = 8$ h was 590 dpm/μg so that:

$$\frac{kt}{2.303} = \log \frac{935}{590} \text{ and } k = 0.058\ h^{-1} \text{ and } t_{1/2} = \frac{\ln 2}{0.058} = 12 \text{ h}$$

decreases from 31,000 dpm μg^{-1} in seeds killed immediately after application of [^{14}C]IAA to 5,400 dpm μg^{-1} in seeds killed 8 h after application of [^{14}C]IAA, thus proving that new IAA is being produced. A plot of the log of specific radioactivity as a function of time of incubation is shown in Fig. 1 and is approximately linear. Without regard as to the source, or sources, of IAA or the pathway(s) of IAA loss, the decrease in specific radioactivity behaves as a first order reaction. Table I also shows a first order rate constant for IAA loss and that in 3.2 h all of the free IAA in the endosperm is "lost" and replaced by new IAA. Since the pool size is 308 pmol kernel^{-1}, new IAA, from all sources, is:

$$\frac{308 \text{ pmol}}{3.2 \text{ h}} = 96 \text{ pmol endosperm}^{-1} \text{ h}^{-1}$$

LOSS OF IAA FROM ENDOSPERM

The amount, or pool, of IAA in the endosperm remains relatively constant as shown in Figure 2 and in reference 35. We have observed in many experiments that fresh seeds increase in IAA for 2 days and then stay constant whereas old seeds decrease (to the same value) and then stay constant. Thus, a loss of some 96 pmol h^{-1} of IAA must occur since that is the amount being made. We previously observed that transport of free IAA out of the endosperm and into the shoot amounted to only 0.015 pmol seedling^{-1} (17, 24). The rate at which IAA is being decarboxylated was studied by the application of IAA labeled in the carboxyl carbon with ^{14}C. These results are presented in Table II and show

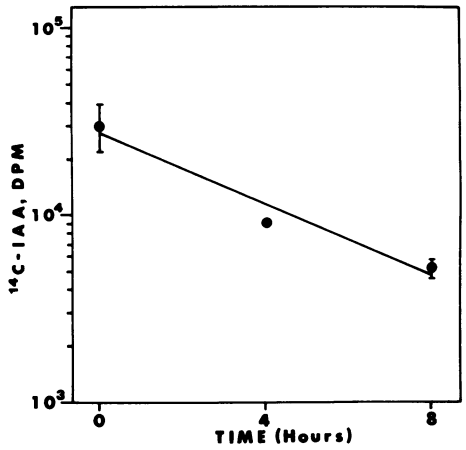

FIG. 1. Change of specific radioactivity (dpm μg^{-1}) of IAA as a function of time of incubation. [^{14}C]IAA was applied to the endosperm at $t = 0$ and reisolated at $t = 0$, 4, or 8 h.

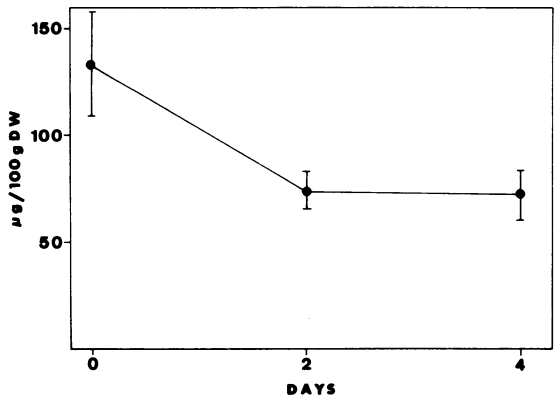

FIG. 2. Amount of free IAA in corn kernel tissues as a function of days of germination. Values are expressed ±SD for six replicate experiments.

Table II. *Decarboxylation of [1-^{14}C]IAA by Zea Kernels*

A total of 28 ng [1-^{14}C]IAA (20,750 dpm) was applied to each seedling. The efficiency of combustion, trapping, and counting was 69% owing mainly to quenching by the base used for trapping. The length of the experiment was 8 h and the apparent pool size of IAA at 4 h was 175 µg so the specific radioactivity of the applied IAA was 20,750/175 + 28 ng = 102 dpm/µg. Thus, 1800 dpm in 8 h was 225 dpm shoot^{-1} h^{-1}/102 dpm ng^{-1} = 12 pmol plant^{-1} h^{-1}.

No. of Experiments	CO_2	Tissue	Total	Decarboxylation
		dpm		%
9	1800 ± 700	11,400 ± 3200	13,200 ± 3500	14 ± 4

that only 12 pmol of IAA h^{-1} endosperm^{-1} are decarboxylated. Thus, 83 pmol h^{-1} (80 pmol of IAA from IAA esters + 15 pmol of IAA from tryptophan −12 pmol h^{-1} decarboxylated) are converted to non-Ehmann reagent-reactive products without loss of the carboxyl carbon. We have previously described some of the chromatographic properties of these products (20).

SOURCE OF ENDOSPERM IAA

IAA Esters. Since all indolylic compounds—those reacting with Salkowski, Ehrlich or the Ehmann reagent—of the kernels of *Z. mays* have been identified (1, 21, 26, 36, 37) and their concentrations determined (ref. 32 and unpublished data), it should be possible to determine which, if any, indolylic endosperm constituent is disappearing at a rate of about 100 pmol h^{-1}, a rate sufficient to supply the newly formed IAA. Previous studies from this laboratory (35) and a recent reexamination (unpublished data) established that all of the esterified IAA of the endosperm, both the A fraction (IAA-glucan) and the B fraction (IAA-inositols) were converted into non-Ehmann-reactive substances at a rate of approximately 1% h^{-1} so that IAA ester concentration in the endosperm declined by 90% in 96 h of germination. The total amount of IAA esters in 4-day-germinated kernels is about four times the IAA-*myo*-inositol content of 6,800 pmol kernel^{-1}. A 1% decrease h^{-1} would amount to (0.01 × 6,800 × 4), or 270 pmol h^{-1}. We surmise that it is the nontransportable, high mol wt IAA-glucan which is hydrolyzed in the endosperm. It constitutes approximately one-half of the ester pool. Thus, 135 pmol IAA-glucan is disappearing from each endosperm in 1 h and this would provide 1.4 times as much IAA as is being formed in each endosperm. We conclude that IAA esters are the source of endosperm IAA. Inasmuch as only one of the many different esters of the endosperm is available as a labeled compound (24), it is at present impossible to study hydrolysis of each ester separately.

Tryptophan, Tryptamine, and Tryptamine Conjugates Are Not Major Sources of Endosperm IAA. The specific radioactivity of [^{3}H]tryptophan immediately and 8 h after application to the endosperm is shown in Table I. As can be seen the specific radioactivity decreases from an initial 15,200 dpm μg^{-1} to 5,060 dpm μg^{-1}. The pool size at zero time was 87,000 pmol kernel^{-1}. We did not determine whether the pool size remained constant but on the assumption that it did, the first order rate constant for tryptophan turnover would be 0.14 h^{-1} and $t_{1/2}$ would be 5.0 h.

Table III shows that the pool size of tryptamine was determined to be 160 pmol kernel^{-1}. Since the pool size was only one-half that of IAA (sufficient for only a 1.5-h IAA turnover period), and since we had previously found only traces of tryptamine conjugates (11), neither tryptamine nor its conjugates could be the source of the newly formed IAA.

Nonenzymic Conversion of Labeled Tryptophan to IAA. Tritiated tryptophan (27.5 Ci/mmol) was chromatographed by TLC using the A solvent of Labarca *et al.* (21). Migration of tryptophan

Table III. *Amount of Tryptamine in 4-day-germinated Kernels of Z. mays*

Experiment[a]	No. of Kernels	Specific Radioactivity of Reisolated Tryptamine	Amount of Tryptamine	
		dpm/μg	*pmol·kernel*$^{-1}$	
1	3620	929,000	128	160
2	4140	555,000	192	

[a] In both experiments, 4.76 μg of tryptamine-HCl with an initial specific radioactivity of 1.86×10^7 dpm was added to the acetone-water seed homogenate. Then, for example in experiment 1;

$$Y = \frac{\left(\frac{1.86 \times 10^7}{9.29 \times 10^5} - 1\right)(4.76)}{3{,}620} = 2.5 \times 10^{-2}\ \mu\text{g kernel}^{-1} \text{ or } Y = 128 \text{ pmol kernel}^{-1}$$

is 20% of that of IAA in this solvent so the resultant tryptophan after elution with 50% 2-propanol was free of IAA. The eluted tryptophan was then dried in a glass, round bottom flask at 12 mm Hg and a bath temperature of 50 C. Rechromatography of an aliquot of the eluate in the same solvent and under identical conditions now showed that 4,480/13,860 cpm or 32% were at the R_F of IAA. Thus, simply drying high specific radioactivity tryptophan *in vacuo* is sufficient to convert it in 30% yield to putative IAA. This large radiological decomposition of tryptophan to IAA is usually not taken into account. In our studies we are multiplying small amounts of radioactivity converted from tryptophan to IAA and thus nonenzymic conversion could account for some of the conversion of [^{3}H]tryptophan to ^{3}H-IAA.

FORMATION OF IAA-*MYO*-INOSITOL IN ENDOSPERM

The pool size and turnover time of IAA-*myo*-inositol are shown in Table I. As can be seen the amount of IAA in the endosperm of a 4-day-germinated seedling is 6,800 pmol and this pool of IAA-*myo*-inositol is replaced every 12 h. The flux is 6,800/12 = 566 pmol h^{-1}. This is in excess of the flux required to provide IAA for the endosperm—even ignoring the formation of IAA by hydrolysis of IAA-glucan. We conclude that IAA-*myo*-inositol in the endosperm is being "diluted" in some unknown way. There are two obvious possibilities: (*a*) transacylation of IAA from IAA-glucan to *m*yo-inositol; or (*b*) IAA-*myo*-inositol glycosides may be hydrolyzed to IAA-*myo*-inositol and then converted back to the glycoside. This would imply a glycosylation function for the IAA-*myo*-inositol glycosides (1, 19).

DISCUSSION

These studies were initiated with the objective of determining pool size and turnover of the indolylic compounds of the seed so that the rate at which indolylic compounds are transported from seed to shoot might be determined. This objective was accomplished and we conclude that IAA-*myo*-inositol and not IAA or tryptophan-derived IAA serves as the source of IAA for the seedling (17, 24). In addition, however, we found: (*a*) that the IAA of the seed is turning over rapidly; (*b*) that most of the metabolized IAA goes to unidentified products; (*c*) that the endosperm IAA is formed by ester hydrolysis—and not from tryptophan or tryptamine; and (*d*) that IAA-*myo*-inositol of the endosperm is being diluted at a rate that suggests it is in metabolic equilibrium with the IAA-*myo*-inositol glycosides (Fig. 3).

Free IAA in the endosperm of kernels of *Z. mays* is subjected to metabolic turnover, being made and destroyed at a rate of 96 pmol seed^{-1} h^{-1} (Fig. 3). The pool size of IAA remains relatively constant at 308 pmol seed^{-1} during 4 days of germination (ref. 36 and Fig. 2). We previously demonstrated (ref. 17 and this paper) that free IAA is being transported out of the seed and into the shoot at only 0.015 pmol h^{-1} and we now show that only 12 pmol h^{-1} are being decarboxylated. Davies (10) has previously observed a small loss of IAA by decarboxylation. Thus, the free IAA in the seed is being converted into, as yet, unidentified metabolites at a rate of 83 pmol h^{-1}. There have been reports (18) of effects of IAA on seed germination but this is the first demonstration of a turnover of IAA in the seed and it is reasonable that this rapid turnover indicates a metabolic function for IAA in the germinating seed.

In three experiments tryptophan was found to be converted to IAA at rates of 22, 25, and 100 pmol endosperm^{-1} h^{-1}. These values are probably high since the tryptophan pool is large and the small amounts of [^{3}H]IAA must be multiplied by large factors to account for dilution of the isotope. Further, we found that simply drying [^{3}H]tryptophan *in vacuo* is sufficient to convert it in 30% yield to IAA by radiological decomposition so that nonenzymic production of [^{3}H]IAA from [^{3}H]tryptophan is difficult to prevent. For these reasons we regard our value for conversion of tryptophan to IAA of 15 pmol seedling^{-1} h^{-1} to be an overestimate. The pool size of tryptamine is 160 pmol seed^{-1} and thus could only supply new IAA to the seed for 1.5 h. *N*-Ferulyl tryptamine and *N*-(*p*-coumaryl)-tryptamine have been found in *Zea* kernels but they are present in only minute amounts (11). Thus, conversion of tryptophan to IAA cannot account for more than 10–25% of the IAA being produced in the endosperm. Our studies apply only to seedling *Zea* still dependent upon endosperm reserves, and are not relevant to prior studies of conversion of tryptophan to IAA in autotrophic plants (30).

IAA esters of the endosperm, unlike IAA, do not remain constant in amount but decline at a rate of 270 pmol endosperm^{-1} h^{-1}. If only the IAA-glucan (28) is being hydrolyzed this would supply 135 pmol endosperm^{-1} h^{-1} of free IAA and this is more than sufficient to supply the 96 pmol h^{-1} of new IAA appearing. We feel that this approximate agreement of rate of ester disappearance and rate of IAA appearance, together with the inadequate rate of tryptophan conversion to IAA, establishes that hydrolysis of IAA esters accounts for most of the endosperm IAA. There is evidence for the occurrence of enzymes that hydrolyze IAA esters to IAA in the seedlings of *Z. mays* (1) and thus a

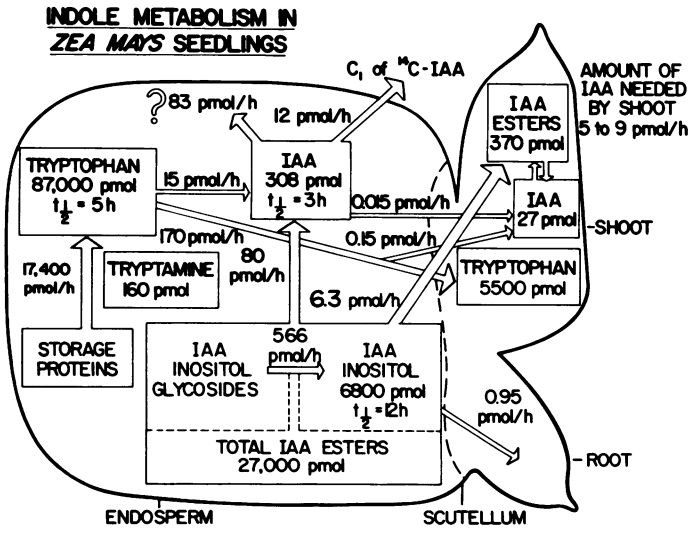

FIG. 3. Pool sizes and rates of turnover of the indolylic compounds in the kernels of *Z. mays* after 4 days of germination. Pool sizes are shown in boxes; the rate at which one compound is converted to another is shown beside the appropriate arrow. Rates of transport are shown alongside the arrows leading from endosperm to shoot or root. Details are described in the text and data, not original to this paper, are from references 2, 17, 24, 27, and 35.

pathway for IAA ester metabolism in endosperm is: IAA ester → IAA → unknown IAA metabolites. Hydrolysis of IAA esters to IAA in the endosperm would be in addition to the transport of unhydrolyzed IAA-*myo*-inositol from endosperm to shoot at a rate of 6 pmol h^{-1} and transport to the root at a rate of 3 pmol h^{-1} (17, 24).

Developmental studies (ref. 9 and unpublished data), *in vivo* labeling experiments (20), and *in vitro* enzyme studies (ref. 20, and Schulze and Bandurski, unpublished) indicate that the IAA esters are synthesized when the kernels are maturing and are hydrolyzed during germination (ref. 36 and this paper).

In this study we find that the turnover of the IAA-*myo*-inositols is more rapid than the rate at which IAA is being produced. Further, the IAA of the endosperm might be coming predominantly from hydrolysis of the IAA-glucan (27) and not IAA-*myo*-inositol. It seems reasonable that the IAA-*myo*-inositols are being made by hydrolysis of the sugar moiety from the 5-position of the inositol of the IAA-*myo*-inositol arabinoside and galactoside (36, 37). Inasmuch as both the IAA-*myo*-inositol pool and the IAA-*myo*-inositol glycoside pools are decreasing in amount, and at about the same rate, it must be that the sugar moiety is coming off and being put back on. This would suggest a role in glycosylation reactions for the IAA-*myo*-inositol glycosides. There is precedence for such a role. Tanner (33) has shown that galactinol is a glycosylation reagent in raffinose and stachyose biosynthesis. Naccarato *et al.* (23) have shown that 5-*O*-galactosyl inositol is the galactosyl donor in lactose biosynthesis, and Kemp (19) has shown that a glucosyl inositol is the glucosyl donor for callose biosynthesis. We postulate that the IAA-*myo*-inositol glycosides play a role in glycosylation reactions. An alternative possibility is that glycosylation acts as a regulatory system for control of the concentration of IAA-*myo*-inositol so that removal of the sugar would be simple hydrolysis and not by glycosyl transfer.

This is the first attempt to make a quantitative study of the dynamics of indole metabolism and turnover in a plant. Each data point required a lengthy isolation procedure to permit determination of the specific activity of the metabolite so that data points are available for only 0, 4, and 8 h. Further, certain assumptions were made. They are: (*a*) that pool sizes remain constant; (*b*) that reactions were unidirectional; (*c*) that reactions obeyed first order kinetics; and (*d*) that the pools were uniform and not multicompartmented. These assumptions were discussed by Broda (6), by Sprinson and Rittenberg (32), and by Zilversmit *et al.* (39) and are the common assumptions made in whole-organism metabolic studies. The ester pool size continuously decreases but this would be at a rate of 1% h^{-1} or only 8% for the 8-h period of this experiment (36). For IAA, we do show that the pool size remains constant and that the reaction is exponential so that assumptions (*a*) and (*c*) appear to be correct. With regard to assumption (*b*) that of unidirectionality, we are unaware of a case where tryptophan is synthesized from IAA in plants and we know that IAA is not converted back to ester in germinating seeds (20) since IAA ester synthesis occurs mainly in developing seeds (ref. 9 and unpublished data) while ester hydrolysis predominates in germinating seeds (35). The last assumption, of pool homogeneity, is most troublesome since the endosperm liquifies more near the scutellar surface and in the area proximate to the seed coats. We studied the rapidity with which dyes colored the endosperm tissue and observed reasonably uniform coloration within 1 h following dye application. Further, the 4-h value for the specific radioactivity of IAA shows that mixing was rapid relative to the length of the experiment since the reaction showed first order kinetics. Ultimately, studies with totally liquid endosperms and directed at single components of the seed indole system must provide more detailed data.

Substrate level metabolism, as for example, sugar, organic acid, or amino acid usage and synthesis, is coupled to the rate at which the organism utilizes substrate (22). The special case of hormone metabolism is more complicated in that hormone levels, regulating growth rate, must be increased, or decreased, to accord with the rate at which the environment permits the organism to grow. Hormonal homeostasis must therefore be pitched to high or low levels depending upon whether the environment permits the organism to grow at high or low rates. We recently proposed a mechanism whereby a plant can couple its hormonal homeostatic mechanism to the environment (4). The mechanism requires that the growth-regulating hormone exist in a free (active) and conjugated (inactive) form and that the enzymes hydrolyzing and making the conjugate (1, 4, 16, 20) be changed in activity by the environment. Such control is suggested by our data for photoinhibition of growth in *Zea* shoot tissue (4).

Here, we demonstrate a steady-state system for maintenance of IAA levels in the endosperm of germinating seeds. The metabolic implications are significant since, in seeds, as in shoot tissue, control of germination, and growth, could be accomplished by controlling the rate at which hormone conjugates are made and hydrolyzed.

Acknowledgments—We are indebted to Ms. A. Schulze for advice on isolation procedures, to Dr. J. Nowacki for the [^{14}C]IAA-*myo*-inositol used, to Ms. Rhonda Epstein for her technical assistance, and to Ms. Joanne Di Lucca Schlub and Ms. Rhonda Epstein for their aid in manuscript preparation.

LITERATURE CITED

1. BANDURSKI RS 1978 Chemistry and physiology of *myo*-inositol esters of indole-3-acetic acid. *In* WW Wells, F Eisenberg, eds, Cyclitols and Phosphoinositides. Academic Press, New York, pp 35–54
2. BANDURSKI RS, A SCHULZE 1974 Concentrations of indole-3-acetic acid and its esters in *Avena* and *Zea*. Plant Physiol 54: 257–262
3. BANDURSKI RS, A SCHULZE 1977 Concentrations of indole-3-acetic acid and its derivatives in plants. Plant Physiol 60: 211–213
4. BANDURSKI RS, A SCHULZE, JD COHEN 1977 Photoregulation of the ratio of ester to free indole-3-acetic acid. Biochem Biophys Res Commun 79: 1219–1223
5. BERGER J, GS AVERY 1944 Isolation of an auxin precursor and an auxin (indoleacetic acid) from maize. Am J Bot 31: 199–208
6. BRODA E 1960 Radioactive Isotopes in Biochemistry. Elsevier, New York
7. COHEN JD, RS BANDURSKI 1977 The rapid separation and automated analysis of indole-3-acetic acid and its derivatives. Plant Physiol 59: S–10
8. COHEN JD, RS BANDURSKI 1978 The bound auxins: protection of indole-3-acetic acid from peroxidase-catalyzed oxidation. Planta 139: 203–206
9. CORCUERA L 1967 Estudio de indolacetilinositoles en granos de maiz. BS thesis, Universidad Catolica de Chile; Santiago, Chile
10. DAVIES PJ 1973 The uptake and fractional distribution of differentially labeled indoleacetic acid in light grown stems. Physiol Plant 28: 95–100
11. EHMANN A 1974 *N*-(*p*-coumaryl)-tryptamine and *N*-ferulyl-tryptamine in kernels of *Zea mays*. Phytochemistry 13: 1979–1983
12. EHMANN A 1977 The Van Urk-Salkowski reagent—a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J Chromatogr 132: 267–276
13. EHMANN A, RS BANDURSKI 1972 Purification of indole-3-acetic acid *myo*-inositol esters on polystyrene-divinylbenzene resins. J Chromatogr 72: 61–70
14. EPSTEIN E, S LAVEE 1975 Uptake and fate of IAA in apple callus tissue using IAA-1-^{14}C. Plant Cell Physiol 16: 553–561
15. HAAGEN-SMIT AJ, WP LEECH, WR BERGEN 1942 Estimation, isolation and identification of auxins in plant material. Am J Bot 29: 500–506
16. HAMILTON RH, RS BANDURSKI, BH GRIGSBY 1961 Isolation of indole-3-acetic acid from corn kernels and etiolated corn seedlings. Plant Physiol 36: 354–359
17. HALL PL, RS BANDURSKI 1978 Movement of indole-3-acetic acid and tryptophan-derived indole-3-acetic acid from the endosperm to the shoot of *Zea mays* L. Plant Physiol 61: 425–429
18. KAHN AA 1977 Seed dormancy: changing concepts and theories. *In* AA Kahn, ed, The Physiology and Biochemistry of Seed Dormancy and Germination. North Holland Publ Co, Amsterdam, pp 29–50
19. KEMP J, BC LOUGHMAN 1978 The role of inositol glucosides in the synthesis of β1,3-glucans. *In* WW Wells, F Eisenberg, eds, Cyclitols and Phosphoinositides. Academic Press, New York pp 439–449
20. KOPCEWICZ J, A EHMANN, RS BANDURSKI 1974 Enzymatic esterification of indole-3-acetic acid to *myo*-inositol and glucose. Plant Physiol 54: 846–851
21. LABARCA C, PB NICHOLLS, RS BANDURSKI 1966 A partial characterization of indoleacetylinositols from *Zea mays*. Biochem Biophys Res Commun 20: 641–646
22. MONOD J, J WYMAN, JP CHANGEUX 1965 On the nature of allosteric transitions: a plausible model. J Mol Biol 12: 88–118.

23. NACCARATO WF, RE RAY, WW WELLS 1975 Characterization and tissue distribution of 6-O-β-D-galactopyranosyl *myo*-inositol in the rat. J Biol Chem 250: 1872–1876
24. NOWACKI J, RS BANDURSKI 1980 *Myo*-inositol esters of indole-3-acetic acid as seed auxin precursors of *Zea mays* L. Plant Physiol 65: 422–427
25. NOWACKI J, JD COHEN, RS BANDURSKI 1978 Synthesis of ^{14}C-indole-3-acetyl-*myo*-inositol. J Label Comp Radiopharmaceut 15: 325–329
26. PISKORNIK Z 1975 Distribution of bound auxin kernels of sweet corn (*Zea mays* L.). Acta Biol Cracoviensia Bot Ser 18: 1–12
27. PISKORNIK Z, RS BANDURSKI 1972 Purification and partial characterization of a glucan containing indole-3-acetic acid. Plant Physiol 50: 176–182
28. RAYLE DL, WK PURVES 1967 Conversion of indole-3-ethanol to indole-3-acetic acid in cucumber seedling shoots. Plant Physiol 42: 1091–1093
29. RITTENBERG D, GL FOSTER 1940 A new procedure for quantitative analysis by isotope dilution with application to the determination of amino acids and fatty acids. J Biol Chem 133: 737–744
30. SCHNEIDER EA, F WIGHTMAN 1974 Metabolism of auxin in higher plants. Annu Rev Plant Physiol 25: 487–513
31. SHERWIN JE, WK PURVES 1969 Tryptophan as an auxin precursor in cucumber seedlings. Plant Physiol 44: 1303–1309
32. SPRINSON DB, D RITTENBERG 1949 The rate of interaction of the amino acids of the diet with the tissue proteins. J Clin Invest 28: 715–726
33. TANNER W 1969 The function of *myo*-inositol glycosides in yeasts and higher plants. Ann NY Acad Sci 165: 726–742
34. THIMANN KV, M GROCHOWSKA 1968 The role of tryptophan and tryptamine as IAA precursors. *In* F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa, pp 231–242
35. UEDA M, RS BANDURSKI 1969 A quantitative estimation of alkali-labile indole-3-acetic acid compounds in dormant and germinating maize kernels. Plant Physiol 44: 1175–1181
36. UEDA M, RS BANDURSKI 1974 Structure of indole-3-acetic acid myoinositol esters and pentamethyl-myoinositols. Phytochemistry 13: 243–253
37. UEDA M, A EHMANN RS BANDURSKI 1970 Gas-liquid chromatographic analysis of indole-3-acetic acid myoinositol esters in maize kernels. Plant Physiol 46: 715–719
38. VAN OVERBEELE J 1936 Growth hormone and mesocotyl growth. Rec Trav Bot Néerl 33: 333–340
39. ZILVERSMIT DB, C ENTERMAN, MC FISHLER 1943 On the calculation of "turnover rate" from experiments involving the use of labeling agents. J Gen Physiol 26: 325–331