Plant Physiol. (1988) 87, 751–756
0032-0889/88/87/0000/00/$01.00/0

Asymmetric Distribution of Glucose and Indole-3-Acetyl-*myo*-Inositol in Geostimulated *Zea mays* Seedlings[1]

Received for publication October 20, 1987 and in revised form January 26, 1988

YOSHIE S. MOMONOKI[2]
Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824-1312

ABSTRACT

Indole-3-acetyl-*myo*-inositol occurs in both the kernel and vegetative shoot of germinating *Zea mays* seedlings. The effect of a gravitational stimulus on the transport of [^{3}H]-5-indole-3-acetyl-*myo*-inositol and [U-^{14}C]-D-glucose from the kernel to the seedling shoot was studied. Both labeled glucose and labeled indole-3-acetyl-*myo*-inositol become asymmetrically distributed in the mesocotyl cortex of the shoot with more radioactivity occurring in the bottom half of a horizontally placed seedling. Asymmetric distribution of [^{3}H]indole-3-acetic acid, derived from the applied [^{3}H]indole-3-acetyl-*myo*-inositol, occurred more rapidly than distribution of total ^{3}H-radioactivity. These findings demonstrate that the gravitational stimulus can induce an asymmetric distribution of substances being transported from kernel to shoot. They also indicate that, in addition to the transport asymmetry, gravity affects the steady state amount of indole-3-acetic acid derived from indole-3-acetyl-*myo*-inositol.

That gravistimulation can induce an asymmetric distribution of IAA is known for several kinds of plants and from several kinds of experiments. Endogenous growth hormone, as measured by exodiffusion and bioassay, shows hormone asymmetry in *Lupinus*, *Avena*, *Zea*, and *Helianthus* (11, 12, 21, 26) with more hormone diffusing from the lower side of a horizontally placed shoot. Exogenously applied [^{14}C]IAA also becomes asymmetrically distributed following application to the tip of a geostimulated shoot (15, 18) or root (17, 20). Endogenous IAA, as measured by GC/mass spectrometry, also is present in greatest amount in the lower half of a stimulated shoot (2, 3). More recently, it has been shown that endogenous free IAA is asymmetrically distributed in the cortex (cortex plus epidermis) tissue of the mesocotyl of geostimulated seedlings of *Zea mays* (4). Hormone asymmetry is often though not always observed (21).

The asymmetry is explained by a tropic induction of movement of the hormone from the upper to the lower side of the stimulated plant. How the lateral transport occurs, in what tissue, what is being transported, and what controls might be operative in moving compounds from the upper to the lower portion of the shoot is not clear. The objective of this and prior studies from this laboratory (2, 3) has been to obtain a fuller understanding of where and how this asymmetry arises so that we may better understand how plants control their endogenous concentration of IAA.

Gravistimulation also induces other asymmetries. Reducing sugars have been found to be distributed asymmetrically in geostimulated sunflower stems (14), internodes of *Dahlia* (28), and wheat internodes (1, 5) with more sugar found in the lower half of the horizontally placed stem.

In this work, we have studied the effect of horizontal positioning of *Zea* seedlings on the distribution of [U-^{14}C]-[5-^{3}H]IAInos[3] being transported from kernel to shoot. Asymmetric distribution develops for these substances following about 30 min of geostimulation. However, [^{3}H]IAA derived from [5-^{3}H]IAInos shows asymmetry in 15 min. This finding suggests two mechanisms for the induction by gravity of asymmetric distribution of compounds, (a) a transport asymmetry, and (b) some mechanism for altering the steady state amount of IAA derived from IAInos hydrolysis.

MATERIALS AND METHODS

Plant Materials. Kernels of *Zea mays* L. cv Stowell's sweet corn (W. A. Burpee Co., Clinton, IA) were soaked for 24 h in running tap water, rolled in paper towels, placed in a plastic bucket containing several cm of water, and germinated for 4 d in darkness at 25°C.

Application of Radiolabeled Compounds. One-fourth of the kernel was cut off, leaving the scutellum intact. Three μl of [U-^{14}C]-D-glucose (13.5 nCi in 75 pmol, ICN, Pharmaceutical, Inc.) in 50% 2-propanol were applied to a cut endosperm surface. After application of radioactive materials, the seedlings were rerolled in paper towels and incubated for 2 h in darkness prior to gravistimulation.

The transport of [U-^{14}C]-D-glucose from endosperm to mesocotyl was assayed after 1, 2, and 3 h of incubation. Ten mesocotyls excised with a razor blade were placed in a 20 ml scintillation vial with scintillation cocktail for 24 h before the radioactivity was counted with a Beckman LS 7000 scintillation counter. [5-^{3}H]IAInos (27 Ci/mmol) synthesized enzymically (22) was a gift from Dr. J. R. Chisnell. Three μl of [5-^{3}H]IAInos (750 nCi in 28 pmol) were applied to a cut endosperm surface.

For determination of free [^{3}H]IAA-derived from [5-^{3}H]IAInos, 4 μl of [5-^{3}H]IAInos (1000 nCi in 37 pmol) were applied. The

[1] Supported by grants to Professor Robert S. Bandurski from the Life Science Section of the Space Biology program (NASA-NAGW-97, ORD 25796) and the Metabolic Biology Section of the National Science Foundation, PCM 82-04017.

[2] Present address: Faculty of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156 Japan.

[3] Abbreviations: [5-^{3}H]IAInos, [^{3}H]-5-indole-3-acetyl-*myo*-inositol; NPA, *N*-1-naphthyl phthalamic acid; TIBA, 2,3,5-triiodobenzoic acid.

Table I. *Distribution of Radioactivity in Z. mays Mesocotyl Cortex following Application of [U-^{14}C]-D-Glucose to the Endosperm*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated cortex				Nonstimulated cortex			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					15 ± 0.9	(50.2 ± 1.9)	15 ± 1.2	(49.8 ± 1.9)
15	19 ± 1.0	(49.1 ± 1.0)	20 ± 1.7	(50.9 ± 1.0)	18 ± 0.8	(49.7 ± 0.6)	18 ± 0.7	(50.3 ± 0.6)
30	23 ± 1.6	(48.2 ± 1.1)	25 ± 2.4	(51.8 ± 1.1)	21 ± 1.0	(50.2 ± 1.2)	21 ± 1.8	(49.8 ± 1.2)
60	27 ± 2.4	(44.1 ± 2.3)	34 ± 3.3	(55.9 ± 2.3)	25 ± 1.3	(50.1 ± 1.4)	25 ± 1.4	(49.9 ± 1.4)
90	31 ± 1.3	(45.0 ± 0.7)	38 ± 1.2	(55.0 ± 0.7)	30 ± 1.1	(49.7 ± 0.6)	30 ± 1.7	(50.3 ± 0.6)

[a] dpm·g dry wt^{-1} × 10^{-4}, mean of 4 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half + dpm in lower half}} \times 100$, mean of 4 values ± SE.

Table II. *Distribution of Radioactivity in Z. mays Mesocotyl Cortex following Application of [5-^{3}H]IAInos to the Endosperm*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated cortex				Nonstimulated cortex			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					58 ± 6	(50.3 ± 1.0)	57 ± 7	(49.7 ± 1.0)
15	68 ± 5	(49.3 ± 0.3)	70 ± 5	(50.7 ± 0.3)	67 ± 6	(50.4 ± 0.8)	66 ± 6	(49.6 ± 0.8)
30	87 ± 1	(49.4 ± 0.9)	89 ± 2	(50.6 ± 0.9)	75 ± 2	(50.4 ± 1.4)	74 ± 1	(49.6 ± 1.4)
60	103 ± 3	(46.7 ± 1.4)	118 ± 3	(53.3 ± 1.4)	89 ± 1	(50.0 ± 0.7)	89 ± 2	(50.0 ± 0.7)
90	123 ± 3	(46.3 ± 0.9)	143 ± 2	(53.7 ± 0.9)	108 ± 5	(50.2 ± 0.5)	107 ± 5	(49.8 ± 0.5)

[a] dpm·g dry wt^{-1} × 10^{-4}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half + dpm in lower half}} \times 100$, mean of 3 values ± SE.

Table III. *Distribution of Radioactivity in Z. mays Vascular Stele following Application of [5-^{3}H]IAInos to the Endosperm*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated stele				Nonstimulated stele			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					108 ± 2	(49.6 ± 0.4)	110 ± 2	(50.4 ± 0.4)
15	133 ± 11	(49.2 ± 0.5)	137 ± 9	(50.8 ± 0.5)	138 ± 7	(49.8 ± 0.5)	138 ± 4	(50.2 ± 0.5)
30	162 ± 3	(42.8 ± 1.2)	217 ± 8	(57.2 ± 1.2)	176 ± 12	(49.9 ± 1.0)	177 ± 18	(50.1 ± 1.0)
60	182 ± 4	(31.5 ± 1.0)	394 ± 10	(68.5 ± 1.0)	277 ± 10	(49.9 ± 0.6)	277 ± 5	(50.1 ± 0.6)
90	378 ± 3	(42.3 ± 0.6)	507 ± 14	(57.7 ± 0.6)	435 ± 15	(50.2 ± 1.0)	432 ± 22	(49.8 ± 1.0)

[a] dpm·dry wt^{-1} × 10^{-4}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half + dpm in lower half}} \times 100$, mean of 3 values ± SE.

Table IV. *Distribution of Radioactivity in Z. mays Coleoptilar Node following Application of [5-^{3}H]IAInos to the Endosperm*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated node				Nonstimulated node			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					135 ± 2	(49.8 ± 0.4)	136 ± 4	(50.2 ± 0.4)
15	165 ± 4	(48.1 ± 0.8)	178 ± 7	(51.9 ± 0.8)	170 ± 4	(50.1 ± 0.5)	171 ± 9	(49.9 ± 0.5)
30	214 ± 4	(48.5 ± 1.3)	227 ± 8	(51.5 ± 1.3)	220 ± 3	(50.0 ± 0.9)	220 ± 9	(50.0 ± 0.9)
60	293 ± 2	(48.7 ± 0.5)	309 ± 5	(51.3 ± 0.5)	316 ± 13	(50.1 ± 0.9)	316 ± 2	(49.9 ± 0.9)
90	448 ± 13	(47.2 ± 0.4)	501 ± 9	(52.8 ± 0.4)	469 ± 16	(50.2 ± 0.7)	466 ± 24	(49.8 ± 0.7)

[a] dpm·dry wt^{-1} × 10^{-4}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half + dpm in lower half}} \times 100$, mean of 3 values ± SE.

amount of [5-^{3}H]IAInos transported from endosperm to the shoot was assayed after 1, 2, and 3 h. Thirty mesocotyl cortices, without stele, comprising the region 1 to 9 mm below the coleoptilar node, were ground in a mortar and pestle with 3 ml of 80% ethanol and filtered with vacuum through one layer of Whatman No. 2 filter paper. The filtrate was transferred to a 20 ml scintillation vial to which 15 ml of scintillation cocktail was added.

Application of NPA, TIBA, and Ethanol. Twelve nmol of NPA (United States Rubber Co., Naugatuck, CT), 20 nmol of 2,3,5-TIBA (City Chemical Co., Okemos, MI) in 0.2% (v/v) ethanol, or 0.2% ethanol alone was applied to a cut endosperm surface

Table V. *Distribution of [^{3}H]IAA Derived from [5-^{3}H]IAInos in Z. mays Gravistimulated Mesocotyl Cortex*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated cortex				Nonstimulated cortex			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					14 ± 1.2	(49.6 ± 1.7)	14 ± 0.6	(50.4 ± 1.7)
15	14 ± 1.0	(43.8 ± 1.7)	18 ± 0.8	(56.2 ± 1.7)	17 ± 1.1	(50.3 ± 1.1)	16 ± 0.6	(49.7 ± 1.1)
30	18 ± 0.4	(44.7 ± 2.3)	22 ± 1.6	(55.3 ± 2.3)	19 ± 0.9	(50.4 ± 1.5)	19 ± 0.2	(49.6 ± 1.5)
60	28 ± 0.7	(46.7 ± 1.7)	32 ± 1.5	(53.8 ± 1.7)	27 ± 0.2	(49.7 ± 0.7)	28 ± 0.7	(50.3 ± 0.7)
90	37 ± 1.2	(46.2 ± 1.6)	43 ± 4.0	(53.8 ± 1.6)	39 ± 1.1	(49.5 ± 0.9)	40 ± 1.9	(50.5 ± 0.9)

[a] dpm·g dry wt^{-1} × 10^{-4}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half} \pm \text{dpm in lower half}} \times 100$, mean of 3 values ± SE.

Table VI. *Distribution of [^{3}H]IAA Derived from [5-^{3}H]IAInos in Z. mays Gravistimulated Vascular Stele*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated stele				Nonstimulated stele			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					41 ± 1.8	(49.2 ± 1.4)	42 ± 2.1	(50.8 ± 1.4)
15	47 ± 4.9	(49.7 ± 1.1)	48 ± 3.6	(50.3 ± 1.1)	44 ± 1.5	(50.0 ± 1.3)	44 ± 2.9	(50.0 ± 1.3)
30	65 ± 3.0	(48.6 ± 1.0)	68 ± 1.6	(51.4 ± 1.0)	52 ± 1.7	(50.1 ± 0.9)	52 ± 3.1	(49.9 ± 0.9)
60	59 ± 3.5	(46.0 ± 1.4)	69 ± 3.2	(54.0 ± 1.4)	60 ± 1.6	(49.5 ± 1.1)	62 ± 1.7	(50.5 ± 1.1)
90	59 ± 1.0	(48.2 ± 0.8)	63 ± 3.2	(51.8 ± 0.8)	70 ± 3.0	(49.5 ± 1.0)	71 ± 1.0	(50.5 ± 1.0)

[a] dpm·dry wt^{-1} × 10^{-1}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half} + \text{dpm in lower half}} \times 100$, mean of 3 values ± SE.

Table VII. *Distribution of [^{3}H]IAA Derived from [5-^{3}H]IAInos in Z. mays Gravistimulated Coleoptilar Node*

Time of Gravistimulation	Radioactivity in Tissue							
	Gravistimulated node				Nonstimulated node			
	Upper		Lower		Right		Left	
min	*dpm*[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%
0					22 ± 0.6	(49.4 ± 1.1)	22 ± 0.6	(50.6 ± 1.1)
15	14 ± 1.3	(24.6 ± 3.1)	42 ± 3.0	(75.4 ± 3.1)	25 ± 0.3	(49.8 ± 1.3)	25 ± 1.6	(50.2 ± 1.3)
30	20 ± 1.6	(32.2 ± 1.2)	41 ± 1.3	(67.8 ± 1.2)	31 ± 3.6	(50.0 ± 1.2)	30 ± 2.2	(50.0 ± 1.2)
60	25 ± 2.4	(36.6 ± 1.0)	43 ± 2.6	(63.4 ± 1.0)	34 ± 1.7	(49.9 ± 1.3)	34 ± 0.2	(50.1 ± 1.3)
90	35 ± 0.2	(41.8 ± 1.8)	49 ± 0.7	(58.2 ± 1.8)	41 ± 1.6	(50.2 ± 0.9)	41 ± 0.2	(49.8 ± 0.9)

[a] dpm·g dry wt^{-1} × 10^{-4}, mean of 3 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half} + \text{dpm in lower half}} \times 100$, mean of 3 values ± SE.

Table VIII. *Effect of NPA and TIBA on Distribution of [5-^{3}H]IAInos Radioactivity in Z. mays Gravistimulated Tissue*

For each plant, NPA (12 nmol·plant^{-1}) or TIBA (20 nmol·plant^{-1}) or ethanol (0.2%) was applied to the endosperm by application of [5-^{3}H] IAInos. The seedlings were incubated for 2 h and gravistimulated for 1 h.

Treatment	Radioactivity in Tissue								
	Mesocotyl cortex				Coleoptilar node				Coleoptile tip
	Upper		Lower		Upper		Lower		
	dpm[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%	*dpm*
NPA	132 ± 4.2	(49.5 ± 1.1)	135 ± 10.1	(50.5 ± 1.1)	305 ± 3.7	(50.3 ± 0.7)	301 ± 5.0	(49.7 ± 0.7)	102 ± 2.7
TIBA	130 ± 1.7	(50.3 ± 0.8)	128 ± 2.4	(49.7 ± 0.8)	303 ± 6.2	(49.7 ± 1.0)	307 ± 6.6	(50.3 ± 1.0)	80 ± 1.9
Ethanol	115 ± 6.5	(45.1 ± 1.9)	141 ± 2.8	(54.9 ± 1.9)	289 ± 7.6	(48.2 ± 0.9)	311 ± 3.8	(51.8 ± 0.9)	68 ± 1.8
None	120 ± 0.5	(45.9 ± 1.0)	142 ± 5.1	(54.1 ± 1.0)	291 ± 1.5	(48.3 ± 0.6)	311 ± 8.2	(51.7 ± 0.6)	67 ± 1.1

[a] dpm·dry wt^{-1} × 10^{-4}, mean of 4 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half} + \text{dpm in lower half}} \times 100$, mean of 4 values ± SE.

followed by the application of [5-^{3}H]IAInos. After application of radioactive or chemical materials, the seedlings were incubated for 2 h and gravistimulated for 1 h.

Gravistimulation of Seedlings. Gravistimulus was provided under a phototropically inactive green light by pinning the kernel to moistened paper-covered styrofoam sheets with the roots covered with moist paper towels. The styrofoam sheets were then placed horizontally for gravistimulated plants or vertically for controls.

Tissue Harvesting. For harvesting of the cortex, the upper

Table IX. *Effect of NPA and TIBA on Distribution of [^{3}H]IAA Derived from [5-^{3}H]IAInos in Z. mays Gravistimulated Tissue*

For each plant, NPA (12 nmol·plant^{-1}) or TIBA (20 nmol·plant^{-1}) or Ethanol (0.2%) was applied to the endosperm following by application of [5-^{3}H]IAInos. The seedlings were incubated for 2 h and gravistimulated for 1 h.

Treatment	Radioactivity in Tissue								
	Mesocotyl cortex				Coleoptilar node				Coleoptile Tip
	Upper		Lower		Upper		Lower		
	dpm[a]	%[b]	*dpm*	%	*dpm*	%	*dpm*	%	*dpm*
NPA	25 ± 0.9	(49.0 ± 1.2)	26 ± 0.4	(51.0 ± 1.2)	32 ± 1.0	(50.2 ± 0.8)	32 ± 1.0	(49.8 ± 0.8)	55 ± 3.5
TIBA	26 ± 0.8	(46.5 ± 0.9)	30 ± 2.0	(53.5 ± 0.9)	31 ± 1.0	(48.5 ± 0.7)	33 ± 0.8	(51.5 ± 0.7)	45 ± 1.6
Ethanol	28 ± 1.3	(44.0 ± 1.5)	36 ± 1.4	(56.0 ± 1.5)	26 ± 0.4	(36.8 ± 0.8)	45 ± 1.8	(63.2 ± 0.5)	36 ± 1.3
None	28 ± 0.6	(43.8 ± 1.8)	36 ± 1.9	(56.2 ± 1.8)	25 ± 1.1	(35.5 ± 0.8)	45 ± 1.3	(64.5 ± 0.8)	35 ± 0.9

[a] dpm·g dry wt^{-1} × 10^{-4}, mean of 4 values ± SE. [b] $\frac{\text{dpm in lower half}}{\text{dpm in upper half + dpm in lower half}} \times 100$, mean of 4 values ± SE.

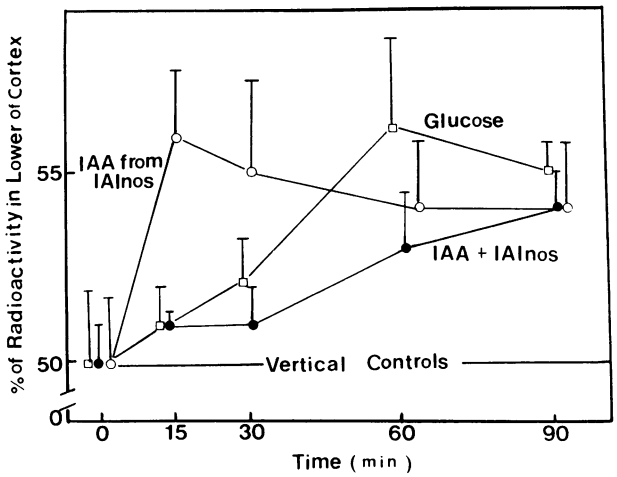

FIG. 1. Percentage of radioactivity in the lower half of the cortex of gravistimulated *Z. mays* seedlings.

portion of the shoot was severed at a point 2 mm below the coleoptilar node, and that portion was discarded. The mesocotyl was then nicked, but not cut through, at a point about 8 mm above the junction between shoot and root. The cortex was then slid off the stele. The 8 mm of cortex closest to the tip were harvested and separated into upper and lower halves. For the vascular stele samples, the mesocotyl from which the upper portion had been severed was cut longitudinally into upper and lower halves. The two parts of the stele were then separated from the cortex with fine surgical forceps. The coleoptilar node was harvested by excising a 4.5 mm region about 2 mm above and below the coleoptilar node and then separating the excised region into upper and lower halves. The coleoptile tip was harvested from the top of coleoptile. The harvested tissues were dropped into beakers standing on dry ice. Thirty to 40 plants (about 15 mg of dry weight) were used for each assay. A green safe light (130 Erg·[cm^2]$^{-1}$·sec^{-1}) was used for all manipulations. As controls, the cortex, stele, and coleoptilar node of nongravistimulated plants were used.

Determination of Radioactivity of [U-^{14}C]-D-Glucose and [5-^{3}H]IAInos. The tissues, frozen on dry ice, were ground in a mortar and pestle in ethanol 1:4 (w/v). The resultant homogenate was filtered with vacuum through Whatman No. 2 filter paper. The filtrate was transferred to a 20 ml scintillation vial and scintillation cocktail was added. The filter, with the residual ethanol-insoluble materials, was dried and weighed. The radioactivity was calculated on a per gram ethanol-insoluble dry weight.

Determination of Free [^{3}H]IAA Derived from [5-^{3}H]IAInos. The tissues, frozen with dry ice, were ground in a mortar and pestle with acetone 3:7 (w/v). The homogenate was allowed to stand for 3 h and filtered. To the filtrate was added a known amount of unlabeled IAA (0.5 μmol) as carrier. The residual acetone-insoluble material was dried, weighed, and used to estimate tissue acetone-insoluble dry weight. The filtrate was acidified with H_3PO_4, and IAA was extracted with $CHCl_3$ (7). The chloroform fractions were dried and dissolved in 50% ethanol and loaded onto a Partisil 10 ODS C_{18} reverse phase HPLC column (0.46 × 25 cm, Whatman). The elution volume where IAA eluted was located with a Gilford-240 Spectrophotometer at 280 nm, and the fractions were pooled and dried *in vacuo*. The dried sample was dissolved in methanol and methylated with diazomethane as previously described (27). The methylated sample was dried in a stream of N_2, and the sample was dissolved in 50% (v/v) aqueous ethanol and loaded on a reverse phase HPLC as previously described. The IAA was eluted with 30% (v/v) aqueous ethanol. The IAA-containing fractions were located by their absorbance at 280 nm, and the radiopurity and identity were checked by TLC on Silica Gel-60 using

Table X. *Summary of Percent of Radioactivity in the Lower Half of Indicated Portion of Gravistimulated Z. mays Seedlings*

Time of Gravistimulation	Portion of Radioactivity in Lower Half of Tissue						
	Mesocotyl cortex			Vascular stele		Coleoptilar node	
	Glucose	^{3}H from [5-^{3}H]IAInos	[^{3}H]IAA from [5-^{3}H]IAInos	^{3}H from [5-^{3}H]IAInos	[^{3}H]IAA from [5-^{3}H]IAInos	^{3}H from [5-^{3}H]IAInos	[^{3}H]IAA from [5-^{3}H]IAInos
min				%			
0							
15	51	51	56	51	50	52	75
30	52	51	55	57	51	52	68
60	56	53	54	69	54	51	63
90	55	54	54	58	52	53	58

$CHCl_3$:CH_3OH:H_2O (85:14:1, v/v) as solvent and were stained with Ehmann's reagent (13). The fractions containing IAA were pooled, and the amount of IAA and the amount of radioactivity in a 1 ml sample were determined by UV absorption at 280 nm and scintillation counting, respectively. The amount of [5-^{3}H] IAInos-derived [^{3}H]IAA was calculated from the following equation:

$$[^3\text{H}]\text{IAA in tissue} = \frac{\text{carrier IAA added}}{\text{carrier IAA recovered}} \times [^3\text{H}]\text{IAA recovered}$$

RESULTS

Distribution of [U-^{14}C]-D-Glucose Radioactivity in the Mesocotyl Cortex of *Z. mays* after gravistimulation. The uptake of [U-^{14}C]-D-glucose into the *Z. mays* shoot increased as a function of time with 204 ± 12 (±SE, n = 4), 367 ± 21, and 546 ± 33 dpm · mesocotyl^{-1} at 1, 2, and 3 h of incubation, respectively. A 2 h preincubation for purposes of loading the tissue was chosen for subsequent experiments. Table I shows the distribution of the [U-^{14}C]-D-glucose in the mesocotyl cortex after the tissues is loaded for 2 h and then gravistimulated for the indicated time. As can be seen, more radioactivity was found in the mesocotyl cortex (upper plus lower) of gravistimulated seedlings than in the (right plus left) controls. The proportion of radioactivity present in the lower half of the mesocotyl cortex increased to about 55% after 1 h and did not change by 90 min. However, asymmetry required more than 30 min to develop. This gravity-induced asymmetric distribution of glucose is in accord with the findings of earlier workers (1, 5, 14, 28). There were no differences in the amount of radiolabel in the right and left halves of control cortices.

Distribution of Radioactivity of [5-^{3}H]IAInos in *Z. mays* Tissues after Gravistimulation. The uptake of [5-^{3}H]IAInos into the *Z. mays* shoot was nearly linear showing 369 ± 51 (±SE, n = 3), 521 ± 37, and 715 ± 32 dpm·8 mm of mesocotyl cortex^{-1} at 1, 2, and 3 h of incubation, respectively.

A 2 h period for loading the tissue with isotope was chosen for subsequent experiments. The distribution of [^{3}H] in the mesocotyl cortex is shown in Table II. An appreciable asymmetry requires more than 0.5 h to develop after gravistimulation. An asymmetric distribution of ^{3}H-radioactivity in the mesocotyl stele appeared after 30 min of gravistimulation when 57% of the label was found in the lower half. This value increased to 69% by 60 min and declined to 58% by 90 min of gravistimulation (Table III). The distribution of ^{3}H-radioactivity in the coleoptilar node was 50, 52, 52, 51, 53% in the lower half after 0, 15, 30, 60, and 90 min of gravistimulation (Table IV). There were no differences in the amount of radiolabel in the right and left halves of control cortices (Table II), vascular steles (Table III), or coleoptilar nodes (Table IV).

Distribution of ^{3}H-Free IAA Derived from [5-^{3}H]IAInos in *Z. mays* Tissues after Gravistimulation. The distribution of [^{3}H] IAA in the mesocotyl cortex during gravistimulation is shown in Table V. Asymmetric distribution in the lower half of the mesocotyl cortex appeared at 15 min, the shortest period tested, then maintained almost the same asymmetry during later gravistimulation periods.

Table VI shows the distribution of [^{3}H]IAA in the mesocotyl stele following gravistimulation. The vascular stele developed an asymmetry during gravistimulation for [^{3}H]IAA. Following 0, 15, 30, 60, and 90 min of gravistimulation, the distribution of [^{3}H]IAA in the vascular stele was 50, 50, 51, 54, and 52%, respectively.

By contrast, the coleoptilar node showed a greater asymmetry of [^{3}H]IAA distribution after gravistimulation (Table VII). The [^{3}H]IAA occurring in the lower half of the coleoptilar node was 50, 75, 68, 63, and 58% of the radioactivity after 0, 15, 30, 60, and 90 min of gravistimulation. Thus, increases and decreases of [^{3}H]IAA in upper and lower halves after gravistimulation occurs slightly in the vascular stele (Table VI) but strongly in the coleoptilar node including vascular tissues (Table VII). There were no differences between right and left halves of the mesocotyl cortex, vascular stele, and coleoptilar node. Again, there were no differences in dry weight between right and left halves of the mesocotyl cortex ($q = 0.92 < 3.15$ [P = 0.05]), vascular stele ($q = 2.71 < 3.15$ [P = 0.05]) and coleoptilar node ($q = 0.34 < 3.15$ [P = 0.05]).

Effect of NPA or TIBA on the Distribution of Radioactivity of [5-^{3}H]IAInos and [^{3}H]IAA Derived from IAInos in the *Z. mays* Gravistimulated Shoot. The distribution of [^{3}H]IAA in gravistimulated shoots after application of NPA or TIBA is shown in Tables VIII and IX. Application of NPA or TIBA inhibits the asymmetric distribution of radioactivity of the [5-^{3}H]IAInos in the mesocotyl cortex and coleoptilar node (Table VIII) and also inhibits the development of a [^{3}H]IAA asymmetry in the mesocotyl cortex and coleoptilar node (Table IX). However, the amount of radioactivity of [5-^{3}H]IAInos (Table VIII) and [^{3}H] IAA (Table IX) in the coleoptile tip was actually higher following NPA or TIBA treatment than in the ethanol or no-treatment controls.

DISCUSSION

A summary of the results of this work is provided in Figure 1 and Table X, which shows the percent of the compound localized in the bottom half of the tissue as a function of time. The data show the following: (a) the radioactivity from [^{14}C]glucose and [^{3}H]IAInos being transported from kernel to shoot becomes asymmetrically distributed in the cortical tissue in 30 to 60 min following the initiation of the geostimulus; (b) the radioactivity in free [^{3}H]IAA, derived from [5-^{3}H]IAInos, is asymmetrically distributed within 15 min following geostimulus; and (c) the greatest asymmetry is that for free [^{3}H]IAA, in the tissue of the node between the coleoptile and mesocotyl and that asymmetry develops within 15 min.

The slowly developing distribution of asymmetries for glucose and IAInos observed in this work correspond well with the period of maximum inequality of growth as described (4). Friedrich (14) has suggested that the asymmetry he observed for reducing sugars was not a direct effect of gravistimulation but was owing to IAA-induced growth. In the present case, however, we are observing an asymmetry of transport and so it would seem that we are seeing either selective leakage from stele to cortex or uniform leakage from stele to cortex followed by lateral migration of radioactivity from the upper to the lower cortical cells. If such is the case, then the lateral transport system can transport glucose just as it is presumed to move IAA.

The asymmetric distribution of [^{3}H]IAA derived from [5-^{3}H] IAInos occurs within 15 min, or less, and thus is faster than the above-described transport asymmetries. This suggests that the [^{3}H]IAA is either being produced more quickly by hydrolysis of [5-^{3}H]IAInos (16) or is being catabolized more slowly (24, 25). We cannot distinguish between these possibilities, but the data do indicate the operation of two mechanisms for attaining unequal distribution of compounds in the mesocotyl: (a) a transport asymmetry and (b) an asymmetry developed by the metabolism of IAA and/or its conjugates.

Last, there is the rapid and large asymmetry shown by the tissues of the node between the coleoptile and mesocotyl. Upward bending in geostimulated dark-grown maize seedlings is initiated in the coleoptile within 1 to 3 min and progresses basipetally to the mesocotyl within 5 min after geostimulation (4). Dayanandan *et al.* (8, 9) found that the leaf-sheath and internodal pulvini are

the primary sites for the upward bending for asymmetry growth after geostimulation, and our work extends and supports that conclusion. Dennison (10) showed asymmetry IAA occurrence in the top and bottom of gravistimulated *Avena* leaf-sheath pulvinus. Additionally, Bandurski's laboratory has established (6, 16, 19, 23) that IAInos diffused from endosperm to the scutellum is taken up into the stele, and IAInos is there hydrolyzed, and then the IAA is transported downward. Thus, it is possible that IAInos which is hydrolyzed in the stele and then moved up quickly into the coleoptilar node could explain asymmetric growth near the node.

Both naphthylphthalamic acid and triiodobenzenzoic acid inhibited the asymmetric accumulation of [^{3}H]IAA formed from [5-^{3}H]IAInos by some as yet unknown mechanisms. This observation if confirmed by a more direct test of the effect of the inhibitors could provide knowledge as to how the so-called transport inhibitors work.

Acknowledgments—I gratefully acknowledge support and advice from Professor R. S. Bandurski. I also wish to thank Dr. J. R. Chisnell for a supply of IAA-inositol.

LITERATURE CITED

1. ARSLAN N, TA BENNET-CLARK 1960 Geotropic behavior of grass nodes. J Exp Bot 11: 1–12
2. BANDURSKI RS 1980 Homeostatic control of concentrations of indole-3-acetic acid. *In* F Skoog, ed, Plant Growth Substances 1979. Springer-Verlag, Heidelberg, pp 37–49
3. BANDURSKI RS, A SCHULZE, W DOMAGALSKI 1986 Possible effects of organelle charge and density on cell metabolism. Adv Space Res 6: 47–54
4. BANDURSKI RS, A SCHULZE, P DAYANANDAN, PB KAUFMAN 1984 Response to gravity by *Zea mays* seedlings. I. Time course of the response. Plant Physiol 74: 284–288
5. BRIDGES IG, MB WILKINS 1974 The role of reducing sugars in the geotropic response of the wheat node. Planta 117: 243–250
6. CHISNELL JR 1984 *Myo*-inositol esters of indole-3-acetic acid are endogenous compounds of *Zea mays* L. shoot tissue. Plant Physiol 74: 278–283
7. COHEN JD, A SCHULZE 1981 Double-standard isotope dilution assay. I. Quantitative assay of indole-3-acetic acid. Anal Biochem 112: 249–257
8. DAYANANDAN P, FV HEBARD, VD BALDWIN, PB KAUFMAN 1977 Structure of gravity-sensitive sheath and internodal pulvini in glass shoots. Am J Bot 64: 1189–1199
9. DAYANANDAN P, PB KAUFMAN 1984 Analysis and significance of gravity-induced asymmetric growth in the grass leaf-sheath pulvinus. Ann Bot 53: 29–44
10. DENNISON DS 1984 Phototropism. *In* Advanced Plant Physiology, MB Wilkins, ed, Pitman Pub Co, Inc, Marshfield, MA, pp 149–162
11. DIJKMAN MJ 1934 Wuchsstoff und geotropische Krümmung bei *Lupinus*. Rec Trav Bot Neerl 31: 391–450
12. DOLK HE 1933 Geotropism and the growth substance. Rec Trav Bot Neerl 33: 509–585
13. 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
14. FRIEDRICH G 1936 Untersuchungen über die Wirkung des natürlichen Wuchsstoffes und der β-Indol-Essigsäure auf den Stoffwechsel der Pflanze. Planta 25: 607–647
15. GILLESPIE B, KV THIMANN 1963 Transport and distribution of auxin during tropic response. I. The lateral migration of auxin in geotropism. Plant Physiol 38: 214–225
16. HALL PJ, RS BANDURSKI 1983 Hydrolysis of [^{3}H]IAA-*myo*-inositol and other esters by extracts of *Zea mays* tissue. Plant Physiol 72: S-115
17. HESTNES A 1979 Distribution of radioactivity from exogenously supplied [1-^{14}C]indole-3-yl-acetic acid and [3,4-^{3}H(N)]gibberellin A_1, in geotropically-stimulated *Picea abies* (L.) Karst, roots. Ann Bot 44: 567–573
18. IWAMI S, Y MASUDA 1976 Distribution of labeled auxin in geotropically stimulated stems of cucumber and pea. Plant Cell Physiol 17: 227–237
19. KOMOSZYNSKI M, RS BANDURSKI 1984 Metabolism of [^{3}H]-5-indole-3-acetyl-*myo*-inositol-[^{14}C]-U-galactose by seedlings of *Zea mays*. Plant Physiol 75: S-108
20. KONING H 1965 The effect of gravity on the transverse distribution of auxin in pea roots. Plant Physiol 40: S-XXXII
21. MERTENS R, EW WEILER 1983 Kinetic studies on the redistribution of endogenous growth regulators in gravireacting plant organs. Planta 158: 339–348
22. MICHALCZUK L, JR CHISNELL 1982 Enzymatic synthesis of 5-^{3}H-indole-3-acetic acid and 5-^{3}H-indole-3-acetyl-*myo*-inositol from 5-^{3}H-L-tryptophan. J Labelled Compd Radiopharm 19: 121–128
23. 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
24. REINECKE DM, RS BANDURSKI 1981 Metabolic conversion of ^{14}C-indole-3-acetic acid to ^{14}C-oxindole-3-acetic acid. Biochem Biophys Res Commun 103: 429–433
25. REINECKE DM, RS BANDURSKI 1983 Oxindole-3-acetic acid, an indole-3-acetic acid catabolite in *Zea mays*. Plant Physiol 71: 211–213
26. RUGE U 1941 Über die geotropische Aufkrümmung decapitierter *Helianthus*-Keimlinge. Planta 32: 176–186
27. SCHLENK H, JL GELLERMAN 1960 Esterification of fatty acid with diazomethane on a small scale. Anal Chem 32: 1412–1414
28. WARNER TH 1928 Uber den Einfluss der geotropischen Reizung auf den Zucker-und Säuregehalt von Sprossen. Jahrb Wiss Bot 68: 431–497