

Action of Gravity on Basipetal Transport of Auxin^{1, 2}

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Materials and Methods

On the basis of tests with over 20 species of erect plants, we reported in 1962 (9) that a strong growth curvature develops in the axis when an erect plant is rotated slowly on a horizontal clinostat. A differential transport of endogenous auxin was indicated by the production of a similar, predictable curvature after the application of triiodobenzoic acid (TIBA) to 1 side of the immature axial tissues. This treatment, known to block the transport (7, 13) of indoleacetic acid (IAA), allowed the axis to grow more rapidly on the opposite side but only when the effects of gravity were eliminated by continuous rotation on a clinostat.

A similar growth curvature in a rotated plant was obtained by removing the leaves from 1 side of the growth zone of the axis. Subsequent growth was slower on the defoliated side. However, in all cases of TIBA blocks and partial defoliation the apparent imbalance of growth regulator in the elongating internodes was prevented or, if curvature had just appeared, corrected when the plant was allowed to grow for a day or 2 in its normal, erect position.

The same results from elimination of unidirectional gravitation action were observed in defoliated and disbudded axes when growth hormone was supplied in solutions or emulsions of IAA. Even with a symmetrical application of this auxin to the cut end of the axis, its elongation was asymmetrical if growth took place on a horizontal clinostat. Gravity appeared to be a factor in maintaining an even distribution of growth regulator within the growth zone of the plant's axis.

The need for more information on the basipetal movement of auxin within the axial tissues has led us to examine the actual distribution of the hormone within the growing stems, with and without the action of gravitational force along the axis. After bioassays of residual, endogenous auxin in growth curvatures failed to reveal an imbalance of growth regulator, a series of tests with radioactive IAA provided evidence for its uneven distribution through lack of the action of gravity on auxin transport down the stem. Other tests have shown a deleterious effect on the rate and form of growth of very young plants if they grow in the horizontal position.

The principal test plants were grown from an established clone of *Coleus blumei* Benth., propagated by branch cuttings and selected for vigorous growth of axes with lengths of 30 to 45 cm. These plants were rooted in 4-inch pots of fertile loam soil and grown in a greenhouse where supplementary light (mixed fluorescent and incandescent) was supplied for 12 hours per day except during the summer months. Smaller pots of the same soil were used in growing seedlings of *Torenia fournieri* Lind. and tomatoes (*Lycopersicon esculentum* Mill.) for 6-week tests of growth with continuous rotation on clinostats under the same light conditions. Plants used to trace the movement of radioactive IAA from leaves into stems and roots were grown from seeds of bush bean (*Phaseolus vulgaris* L.) and cabbage (*Brassica oleracea* L.).

Clinostats. To remove the possible effects of gravity on auxin movement, plants were attached in sets of 5 (fig 1, 2) to horizontal clinostats with rate of rotation either 1 or 10 rph. The horizontal axis of each plant was only about 10 cm from the center of rotation of the wheel, thus avoiding any possible eccentric effect on axial growth such as that reported by Hoshizaki and Hamner (6). The curvatures in the *Colcus* stems were equally variable with the 2 rates of rotation, neither of which induced sinusoidal vibrations that might have developed small centrifugal forces in the plant axes (8).

The horizontal orientation of these axes eliminated longitudinal action of gravitational force within them. The omnilateral stimulus of gravity on the rotating stems could not be avoided but the continual change in position of the immature tissues prevented stimulation of the geotropic mechanism; the presentation time was always less than that required. It was therefore possible to grow control plants in which there had been no action or effect of gravity on the transport of auxin within the stems.

Bioassay. The *Avena* curvature method (18) was used to measure the residual auxin in the convex and concave halves of the axis after 18 hours of clinostat growth had produced a curvature in a *Coleus* stem. Equal weights of tissue from the 2 sides were obtained by manual bisection and assayed by identical procedures. Standard techniques were used in preparing the coleoptiles and in measuring the effects of unknown amounts of auxin. Endogenous auxin was extracted in chloroform from the 2 separate lots of axial tissues (10, 15).

¹ Received March 4, 1965.

² This work was supported by research grants from the National Aeronautics and Space Administration (NsG-231) and the National Science Foundation (G-13140).

Radioassay. Radioactive IAA was used to measure imbalance in auxin supply to the convex and concave halves of a curved axis. IAA-2-C¹⁴ (usually with specific activity 4.4×10^5 cpm/mg IAA) in 1% emulsion of lanolin was used to replace the endogenous auxin supply in straight, defoliated, disbudded *Coleus* axes. The auxin paste was usually applied in a layer about 1 mm thick over the cut surface across the first firm node of each *Coleus* stem. As described below, asymmetrical conditions of auxin supply or transport within the stems were provided for special experiments. The same lanolin emulsion was also used to trace auxin movement as far as the root system, with the paste applied through leaf epidermis (10,11) of intact plants.

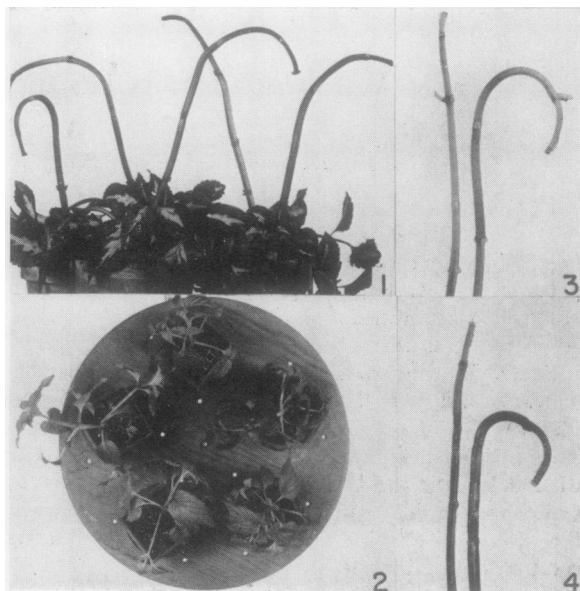
Immediately after any treatment, the control plants were attached to clinostats in a room at a temperature of $22 \pm 1^\circ$ and totally dark except during inspections and care of the erect and horizontal plants. After a period of growth on a clinostat, the curved portion (fig 1) of each of the many control stems was excised below the supply of IAA in lanolin. The curved pieces were taken at once into a 4° refrigerator and bisected into convex and concave halves of equal weights as for bioassay. The corresponding straight stems of plants grown erect to gravity were excised and bisected by the same procedure.

The 2 lots of tissues for any measurement of C¹⁴ were diced and ground in mortars at 4° with chloroform and acid-water at pH 2.8 to 3.0, later with absolute alcohol, to obtain 3 sets of extracts for half of a curved or straight stem, as described in earlier reports from our laboratory (10,11). Small aliquots of the 6 extracts, each concentrated to a small, measured volume, were used to measure radioactivity by means of a Nuclear-Chicago liquid scintillation counter. Data for radiocarbon were computed later for equal volumes of the extracts. The standard errors of the measurements were less than 5%.

Measurements of Intact Plants. Tests of the total effects of gravity on the growth of a seedling plant were designed to compare lengths of internodes, fresh and dry weights of the shoot, and morphology of the plant after it had grown for 6 weeks either erect or on a clinostat at 1 rph. *Torenia* plants were transplanted from seed flat to special pots (fig 2) when 2 true leaves were well expanded at the first node above the cotyledons. Tomatoes were germinated in 3-inch pots that were attached to the clinostat wheels when the cotyledons were supported firmly on the hypocotyls. Other plants of each seedling type grew erect in equal numbers on a greenhouse bench beside the clinostats.

Experimental Results

Axial Curvatures. The characteristic form and variation in degree of the axial curvature developed by *Coleus* during horizontal rotation are seen in fig-



FIGS. 1-4. Axial curvatures in plants grown on horizontal clinostats that rotated at 1 rph. FIG. 1. Defoliated *Coleus* axes with variable curvatures after 24 hours. Auxin from terminal cap of 1% IAA-C¹⁴ in lanolin. FIG. 2. Semi-prostrate *Torenia* plants grown from seedlings for 1 month, as seen in top view. FIG. 3. Defoliated and disbudded *Coleus* axes with lateral supply of 1% IAA-C¹⁴ in lanolin. Stem at left stood erect for 24 hours while stem at right grew on clinostat faster on the IAA side. FIG. 4. Similar *Coleus* axes after 24 hours, each with a terminal cap of 1% IAA-C¹⁴ paste and a lateral block of 1% TIBA in lanolin. Erect stem at left. Clinostat effect at right with curvature toward TIBA side.

ure 1. When the leaves are removed and auxin supplied in terminal caps of lanolin paste, the curvature extends through the 3 youngest internodes and is identical to the curvature of intact fully leaved stems. The total curvature is in the direction that the original axis inclined slightly. Control plants show no curvatures with lanolin free from IAA.

The axial curvatures of rotated *Torenia* plants (fig 2) are similar but are partly obscured in this top view of the plants by the results of leaf and branch epinasty. In contrast with the development of strong *Coleus* curvatures in established internodes within a few hours, the curved axes of these semi-prostrate *Torenia* plants were formed of internodes added during slow growth with continuous rotation. In neither type of growth was there a relation between direction of curvature and the axis of horizontal rotation. The curvatures in seedling tomatoes were smaller and not in the same plane from one internode to another. The determinants of the curvatures appear to be internal, with degree of curvature often proportional to the rate of elongation of internodes.

Bioassays of Auxin in Curvatures. Diffusible auxin could not be obtained in measurable amounts from *Coleus* stems. The results of bioassays of extractable auxin from the convex and concave halves of axial curvatures are shown in table I. From the 6 sets of measurements it appears that in spite of the rapid growth of the convex side of each stem, it held no consistent excess of unaltered hormone over the free auxin in the tissues which had made less growth. The extracts and assays were made after only 18 hours of basipetal transport without the pull of gravity acting parallel to the axis but the curvatures formed quickly as the result of pronounced differences in rates of growth. The growth rates are not reflected in the residual auxin taken from the opposing halves of the axes. These auxin molecules were evidently not involved in controlling the uneven growth of the internodes but they show movement or production of endogenous IAA in both sides of the stem.

Radioassays of Auxin Supply to Curvatures. The radioisotope method for measuring the distribu-

tion of auxin supplied to the growth zone from a symmetrical source on the cut end of the *Coleus* axis provided evidence for an imbalance of growth regulator in favor of the convex half of a curved stem. The data for radioactivity in tables II and III show a predominance of radiocarbon in the extracts from the tissues which had made the greater growth. Regardless of the chemical identities of the C^{14} compounds extracted from tissues by the 3 solvents, each radioactive atom represented a molecule of IAA that had been transported down the plant axis.

The distribution of the radiocarbon among the 3 extracts was always similar to that in auxin studies previously reported in some detail with our extraction method. Most of the radioactivity was carried in degradation compounds soluble in acid-water or ethanol. The chloroform extract held the unaltered IAA. Earlier work with *Coleus* branches (12) and petioles (11) has shown this residual IAA to amount to less than 5% of the auxin supplied from the lanolin paste. The IAA is known to be transported in unaltered form (7, 10, 11, 13) and was degraded

Table I. *Bioassays of Auxin from Axial Curvatures of Coleus*

Test no.	Extract sample g	Degrees <i>Avena</i> curvature		Statistical significance of difference
		Convex side	Concave side	
1	10.32	26.9	27.0	Not significant
2	12.64	11.4	8.5	Not significant
3	22.00	8.8	8.3	Not significant
4	8.18	17.9	15.0	Not significant
5	9.75	15.6	3.9	Highly significant
6	5.31	2.7	2.5	Not significant

Table II. *Radioactivity from C^{14} in Bisected Axial Curvatures of Coleus*

Expt. no.	Hr of expt.	Wt of tissue g	Cpm in 0.020 ml each of 0.50 ml extracts*		Ratio Convex/Concave
			Convex side	Concave side	
1	46	47.83	436.2	310.8	1.403
2	41	39.29	173.8	58.0	2.997
3	42	27.36	80.0	32.6	2.454
4	47	21.34	165.4	82.3	2.010
5	47	15.82	130.2	64.0	2.034
6	47	21.30	91.4	70.6	1.295
7	47	30.75	326.4	106.3	3.069
8	47	35.67	145.4	98.9	1.470
9	47	18.76	43.2	33.7	1.282

M = 2.002 ± .236

* Separate extracts in chloroform, acid-water and ethanol.

Table III. *Interrelations of Curvatures in Coleus Axes and Supply of IAA- C^{14}*

Test no.	Tissue wt in g		Cpm in 0.050 ml each of 0.50 ml extracts			
	Curvature > 90°	Curvature < 90°	Curvature > 90°		Curvature < 90°	
			Convex side	Concave side	Convex side	Concave side
1	21.71	15.86	619.5	433.5	202.3	184.2
2	17.66	14.71	384.7	265.6	160.2	140.2
3	12.58	12.72	225.3	147.9	197.0	164.0

in the tissues before extraction. The unequal distribution of the C^{14} between the opposing halves of the axial curvatures demonstrates the movement of more auxin into the side which made the greater growth in the absence of an effective force of gravity.

Although the mean distribution ratio of radioactivity in table II indicates twice as much IAA supplied to the convex side, the wide variation in the ratio points to the absence of a positive mechanism for transporting an excess of IAA through certain sectors of the stem when the plant is rotated on a clinostat. The excess of auxin in the convex side varied from about 30% to about 200% but these data represent the means for at least 30 stems used in a single analysis. The variations in degrees of curvature for any one experiment (cf. fig 1) and between lots of plants in the 9 sets of measurements were usually even greater. The errors of analysis add to the difficulty of correlating distribution ratios of the IAA with the nature of the curvature.

The data of table III provide some evidence, however, for a rough agreement between the degree of curvature in any one axis and the imbalance of auxin transport to its growth zone. The stronger curvatures with a mean convex/concave ratio of 1.47 proved to contain on the average a greater imbalance of auxin than that in weak curvatures (mean ratio = 1.15) under the same experimental conditions. The differences in absolute amounts of radiocarbon in the opposing types of curvatures seem also to show that more IAA is carried into the growth zones when the curvatures are over 90°.

Transport of a Lateral Supply of IAA. The effect of gravity on the basipetal transport of a strongly asymmetrical supply of IAA- C^{14} was tested on *Coleus* plants with the axes defoliated and disbudded. The paste was applied to 1 side of the axis in a narrow strip 1 cm long, close to the decapitated end (fig 3). Sets of control plants were at once attached to clinostats in a dark room where an equal number (25 or 30) of experimental plants were set upright beside the clinostats.

The result was always straight axes in the erect plants and curvatures on the clinostat where each stem grew more rapidly on the side to which the auxin paste had been applied. Figure 3 shows typi-

cal growth forms for the 2 sets of plants after 24 hours.

Gravity appeared to have corrected the imbalance in auxin supply while an excess of auxin was transported down 1 side of each stem if the plant was turned on a clinostat. The data of table IV show this difference in the distribution of the radioactive auxin below the level of the IAA supply. The transport system alone carried only part of the auxin to tissues in the half of the stem opposite the side with the auxin paste. The distribution of C^{14} was uniform in erect straight stems but there was about 40% (mean = $40 \pm 2\%$) more radiocarbon on the convex than on the concave sides of rotated, curved stems.

Transport of IAA- C^{14} Past Transport Blockage by TIBA. Tests were also made of the capacity of gravity to provide the axis with an even distribution of IAA- C^{14} when the basipetal transport from a terminal, symmetrical supply was interrupted in a sector below the cap of auxin paste. A localized block was created by applying a 1 cm strip of 1% TIBA in lanolin to 1 side of the axis just below the auxin cap on the cut end (fig 4). Groups of *Coleus* plants with this treatment were grown together, half erect and half on clinostats, for nearly 24 hours. Tissues below the TIBA level were then excised, bisected, and ground with solvents to obtain measurements of radiocarbon in extracts from the unblocked and TIBA sides.

The results were visually like those for an unblocked, lateral supply of auxin (cf. fig 3, 4), with curvature produced by more growth on the unblocked side only if the plant was horizontal and rotated. The data for the distribution of radiocarbon (table V) also show essentially the same excess of C^{14} in the convex halves of the curvatures (mean excess = $45 \pm 6\%$) and again no difference in opposing sides of erect, straight stems. Gravity seems to produce an even distribution of growth regulator either through the blockage by TIBA or in the axis below it.

The effect of the TIBA block on the amount of auxin transported past it can also be seen in the data of table V, last line. Only half as much C^{14} was obtained from the curved stems as from the same weight of tissue in the straight stems that were

Table IV. *Transport of a Lateral Supply of IAA- C^{14}*

Expt no.	Hrs of growth	Tissue wt in g		Cpm in 0.050 ml each of 0.50 ml extracts			
		Clinostat plants	Erect plants	On clinostat		Erect to gravity	
				IAA side	Opposite side	IAA side	Opposite side
1*	42.0	12.72	10.61	26.0	19.4	25.7	25.3
2	18.0	9.04	8.65	100.2	71.0	107.4	110.0
3	24.0	7.57	10.07	132.7	96.5	110.2	109.7
4	23.5	11.06	11.32	106.1	74.3	218.0	208.0
5	24.0	11.17	11.10	414.1	285.9	387.9	384.2

* Activity of IAA- C^{14} was 1.1×10^5 cpm/mg but in the other 4 experiments it was 4 times as great.

under the influence of unidirectional gravity. If the data of table V for these erect plants are compared, however, with the corresponding counts of radioactivity for erect stems in table IV, where transport by internal processes was undisturbed, it appears that the TIBA block may have been partially effective deep into the stems; the heavier stems of the TIBA test with normal action of gravity received on the average less than one-third as much IAA as the erect stems without blocks below their auxin supplies. Gravity seems to correct an imbalance due to more interference in 1 sector than another, rather than to undo or offset the inhibitory effects of an agent like TIBA.

Transport of IAA into Roots. The radioisotope method was also used to test the possible action of gravity on the movement of auxin from leaves into the root system. The radioactive compound was applied in 1 % auxin paste to the lower epidermis of mature leaf blades (11) and assayed for radiocarbon in separate extracts from petioles, stems and roots. Experimental plants were set erect in darkness beside the clinostats that rotated the same number of control plants, all supplied with IAA-2-C¹⁴ for nearly 2 days.

The entries in table VI are based on the total radioactivity in aliquots of the triple extraction of each of the 3 plant parts. For comparisons independent of the amounts of radiocarbon extracted in the several tests with bean and cabbage plants, the distribution of radioactivity between the organ

systems is also shown as percentages of total C¹⁴ extracted from each group of plants per test.

The data show that consistently greater amounts of IAA and its nonvolatile degradation products were obtained from the roots when the test plants had stood erect to gravity. The effect was different only in degree for the 2 species. In all cases more than half of the C¹⁴ remained in the petioles where large amounts of it have been shown (11) to be outside the transport system. The higher percentages of radioactivity in the roots of erect plants was associated with lower percentages in the stems through which the radioactive auxin had passed. Only the total C¹⁴ from each organ is shown in table VI but the amounts extracted from the roots represent basipetal movement of IAA, the transport form (4, 10, 11, 12).

Reduced Axial Growth on Clinostats. Seedling plants were used for a limited study of the general effect of clinostat treatment on axial length and shoot development during prolonged deprivation of the action of gravity along the plant's axis. Effects on weight of shoot were also recorded (bar diagrams in fig 5) but some reduction in fresh and dry weights was expected through reduction in photosynthetic efficiency by leaf epinasty.

Both *Torenia* and tomato seedlings developed shorter internodes on clinostats (cf. graphs in fig 5). The mean length of new growth in 10 tomato axes was reduced to less than one-third that of upright plants at every stage during the 6 weeks of

Table V. *Transport of IAA-C¹⁴ Past Lateral Block of TIBA*

Expt no.	Hrs of growth	Tissue wt in g		Cpm in 0.050 ml each of 0.50 ml extracts			
		Clinostat plants	Erect plants	On clinostat		Erect to gravity	
				Free side	TIBA side	Free side	TIBA side
1	23.0	18.17	16.10	45.4	33.4	84.9	87.8
2	26.0	17.98	16.53	17.9	11.5	29.0	28.1
3	23.5	17.14	15.76	19.8	14.7	61.0	58.7
4	23.0	16.44	16.82	65.2	42.5	62.8	65.8
Mean		16.87		36.4	25.0	61.7	62.4

Table VI. *Gravity Effect on Transport of IAA-C¹⁴ from Leaf Blades to Roots*

Extracted tissues	cpm %		Radioactivity in 0.010 ml each of 0.50 ml extracts							
	cpm	%	Plants erect		Plants on clinostats		Plants erect		Plants on clinostats	
			cpm	%	cpm	%	cpm	%	cpm	%
Beans										
Petioles	618.3	65.4	417.2	74.0	870.1	58.3	971.1	53.7	1233.6	72.1
Stems	202.4	21.4	104.4	18.5	270.7	18.1	731.6	40.5	366.3	21.4
Roots	124.9	13.2	42.4	7.5	352.0	23.6	105.1	5.8	111.5	6.5
Total	945.6		564.0		1492.8		1807.8		1711.4	
Cabbage										
Petioles	2406.9	62.5	2834.8	67.0	1508.4	55.4	1522.4	67.5	1522.4	67.5
Stems	1198.1	31.1	1076.0	25.4	1124.2	41.3	703.6	31.2	703.6	31.2
Roots	243.8	6.4	321.0	7.6	89.8	3.3	29.6	1.3	29.6	1.3
Total	3848.8		4231.8		2722.5		2255.6		2255.6	

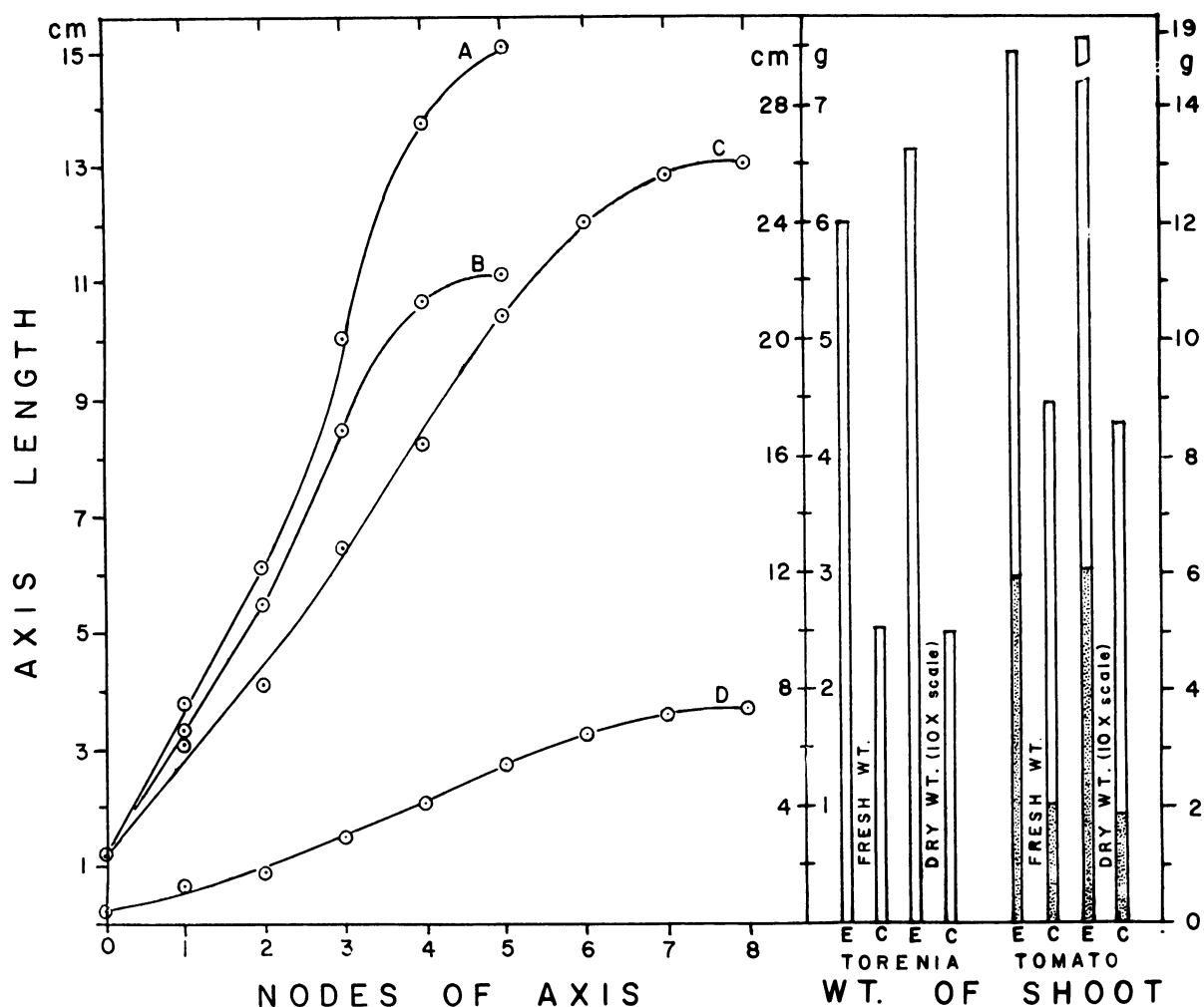


FIG. 5. Effects of growing seedlings either erect or on horizontal clinostats. Graphs A and B show mean lengths of 20 *Torenia* stems each for erect and clinostat positions (scale at left). Graphs C (erect) and D show corresponding means for increase in length of 10 tomato stems (scale at right). Weights of shoots plotted with similar left and right scales. E and C under bars show growth positions. Stem portion of tomato weight shown by stipling.

growth. The effect was produced entirely by failure of internodes of both species to elongate as much in the stunted plants, since the same number of nodes and leaves developed in experimental and control plants. Microscopic measurements of parenchyma cell length in the third internode of tomato plants gave means of 50.8% less elongation of the pith and 33.1% less elongation of the middle cortex in plants grown with a horizontal stem.

Torenia developed fewer nodes than tomato during the same growth period, due to the early production of flower buds which were more numerous (mean of 3.3) on the erect plants than on the clinostat plants (mean of 1.4) at the close of the test. There were also fewer branches of *Torenia* on the clinostats. No tomato plants had buds or branches but the adequate provisions for their growth were

reflected in the taller, heavier tomato plants in up-right pots.

The bar diagrams of figure 5 show that the reduction in weight of *Torenia* plants on clinostats was relatively greater than that for tomato plants, in spite of the relatively shorter axes of tomatoes on clinostats. The difference was associated with somewhat less leaf exposure to light through stronger curvatures in the *Torenia* axes (fig 2) and through more shading of the folded, simple leaves of *Torenia*. The diagrams of figure 5 also show differences in water content of tomato and *Torenia* shoots grown on clinostats but this and other points will receive attention later. The significant evidence for this study of auxin transport is the great reduction in length of internode when the plants were not grown upright.

Discussion

The early work on relations between gravity and growth regulators in stems was indecisive, partly because movements of their auxins could not be monitored. An example of the confusing evidence is to be found in the 1935 report of Brain (2) on growth rates of seedling organs on horizontal clinostats. He found accelerated growth of *Helianthus* hypocotyls and corn coleoptiles. The hypocotyl of *Lupinus* elongated more slowly if the seed was germinated on a clinostat but more rapidly if the early stages of germination had not been rotated.

In his review of 1952, Soding (14) took the position that gravity has no effect on basipetal movement of auxin but later (by personal letter to us in 1959) he expressed his belief that gravity aids this transport in stems. Vendrig (16) reported that it controls the longitudinal transport in *Coleus* stems of a substance which retards abscission of leaves but his methods indicated that the basipetal movement of neither endogenous nor exogenous IAA is affected by gravity. For cases of reduced elongation of stems kept for some time in the horizontal position, as in the work of Wareing and Nasr (17) with woody branches, Vendrig favored indirect control of growth by correlative effects rather than by retarded transport of growth regulators. After surveying the earlier reports of clinostat studies with various seedlings and established plants, he concluded that the longitudinal component of gravity has uncertain effects.

From a later survey, however, with emphasis on the *Avena* coleoptile, Anker (1) accepted decreased coleoptile elongation in the horizontal position as evidence for aid from gravity in basipetal auxin transport but he called for further study of the process because the evidence was not strong.

The present study provides information on auxin movement and its distribution within the growth zone of a typical *Dicotyledonous* stem. The radioassays of our system of extracts supplied quantitative descriptions of distribution of all the radioactive IAA in immature internodes of *Coleus* axes, with and without curvatures. The amounts of unaltered endogenous auxin in the concave and convex halves of axial curvatures proved to be equal and could not, therefore, explain the unequal growth. The corresponding unaltered exogenous IAA-2-C¹⁴ has been shown in our earlier work with epinasty (10, 11) and geotropic curvatures (12) in *Coleus* to appear in small amounts in the chloroform extracts. From this experience with *Coleus* stems and from the low radioactivity (too low for isolation of the IAA) in the chloroform extracts that contributed to table II above, we estimate that no more than 5% of the radioactive IAA had escaped destruction in the axial curvatures. Larger fractions of exogenous IAA were found by Goldsmith and Thimann (3) and by Goldsmith and Wilkins (4) to have been immobilized

in coleoptiles at the end of a few hours. The residual endogenous IAA in axial curvatures of *Coleus* may have been an immobilized fraction but was probably a poor index of the auxin supply that controlled the unequal growth rates.

Visual impressions of highly variable curvatures, like those in figure 1 and others with even greater differences in growth rates on opposite sides of a *Coleus* stem, suggest that there is no regular, internal mechanism for the differences when the plants grow in the horizontal position. There is no such predictable imbalance of growth rates as that demonstrated for branch and leaf epinasty in plants on clinostats (10, 11). The variable ratios of IAA carried into the 2 sides of the curved stem (cf. tables II, III) support this observation. In about 4% of *Coleus* stems tested on clinostats there was no measurable curvature, as though equal amounts of auxin had been delivered to opposing sides of the stem. Measurements showed this to be the case for straight stems in which gravity had corrected experimental imbalances in supply (table IV) or transport capacity at some point (table V).

Without a positive mechanism for imbalance in auxin content on clinostats, differences in efficiency of basipetal transport or in resistance to basipetal movement through transport tissues could produce the same imbalances in auxin supply. The production of stronger curvatures when greater amounts of IAA were moving into equal weights of growing tissue (table III) may be due to a greater inequality in transport because the path of less resistance (or greater transport capacity) carries an even higher percentage of the auxin supply when it is more abundant. We know too little of the transport mechanism to appraise the situation exactly.

With the possibility that differences in their anatomical or physiological capacity for unaided auxin transport in opposing sides of a stem produce curvatures in the absence of the usual effects of gravity on erect stems, the major question remains as to the method by which gravity overcomes such tendencies to unequal growth. From the radioassay evidence for the successful corrective action against unbalanced supply of auxin or uneven transport capacity (tables IV, V), it appears that gravity had moved the excess auxin to the side of the stem which would otherwise have been the concave side of a curvature. It has been shown that gravity does move auxin molecules to the lower side of a *Coleus* stem (12) as it does in coleoptiles (3, 4). The process of maintaining an erect stem need therefore be no more than this lateral transport of auxin in negative geotropism. An incipient curvature would be checked at once by a shift of auxin when it was moved to the slightly lower side. The auxin would be present in the transport tissues, available to lateral movement by gravity. The evidence for this equalized distribution of IAA within the *Coleus* stems, below a zone of unequal concentration, also confirms

other evidence that auxin is chemically unaltered during basipetal transport.

If there is a constant action of gravity to transport some IAA downward, the effect should not be limited to lateral movement in a nonvertical stem. Hertel and Leopold (5) provided evidence for basic similarity in the lateral and basipetal transport of auxin. Goldsmith and Wilkins (4) suggested that the mechanism is the same for both in coleoptiles, with orientation as the determinant of the ratio between lateral and basipetal movement. Gravity may partially offset basipetal transport in inverted stems, as Yamaki (19) reported it to do in *Solanum tuberosum*, but the IAA in the transport tissues of an erect stem must logically be pulled down it.

A test of this condition was made in the radio-carbon experiments with bean and cabbage plants. Some of the unaltered IAA reached the stems and a small percentage went as far as the roots. The data in table VI show that more IAA-C¹⁴ was transported from the stems into the roots when the plants were erect to gravity. The evidence from the shorter internodes of *Torenia* and tomato plants, in stems that were never erect, shows that the elongation of their axial tissues was much retarded by growth in the horizontal position, as though the growth hormone did not move down the stem as rapidly as in an erect axis.

The action of gravity in maintaining the erect form of the terrestrial plant seems therefore to be direct and significant. The evidence supports an interpretation of regulatory effect as mediated through auxin transport. The mechanism of negative geotropism is an important physiological aspect of the evolution of upright terrestrial vegetation.

Summary

Immature plants of *Coleus blumei* Benth. were used to trace the movement of auxin in relation to previously reported curvatures in the axes of plants as they grow on horizontal clinostats. Seedlings of *Torenia fournieri* Lind. and *Lycopersicon esculentum* Mill. and older plants of *Phaseolus vulgaris* L. and *Brassica oleracea* L. were also grown erect and on clinostats in studies of axial growth rates and auxin transport.

Bioassays of endogenous auxin from the convex and concave sides of curvatures in *Coleus* stems showed no significant imbalance in residual growth hormone extractable with chloroform.

Radioassays of the distribution of extractable, radioactive indoleacetic acid (IAA-2-C¹⁴) within a curved stem showed that a large excess of this auxin had been transported from a terminal supply into the convex half of the curvature. The measurements were made from a combination of extractions in chloroform, acid-water and ethanol with most of the radiocarbon held in degradation products of the radioactive compound.

Similar growth curvatures and unequal distributions of radioactivity were obtained when the IAA-C¹⁴ was supplied to 1 side of an axis or when its basipetal transport from a terminal supply was blocked on 1 side close to the supply. These curvatures of stems and the imbalances in auxin distribution did not appear, however, when the *Coleus* plants stood erect to gravity. The evidence from these isotope studies supports the interpretation of the regulatory action of gravity on auxin transport and upright growth as being due to the mechanism of negative geotropism.

Radioassay of the distribution of IAA-C¹⁴ and its degradation products throughout the shoot and roots of bean and cabbage plants showed the movement of more auxin into the roots of erect plants than into those of plants grown on clinostats while the auxin was being absorbed through the leaf epidermis. Less radioactivity remained in the stems of the erect plants.

Seedlings of *Torenia* and tomatoes underwent much reduction in length of internodes and weight of shoot when grown on clinostats for 6 weeks after germination. This retarded elongation of the growth zones without change in the productivity of the apical meristem agrees with the evidence from the older plants of 3 other species that gravity provides direct, significant aid to the basipetal transport of auxin in erect terrestrial plants.

Acknowledgments

The author acknowledges the valuable assistance of Dr. Victor Weeraratne in the bioassay work. He is also indebted to Dr. K. V. Thimann for helpful advice on several points, and to Dr. R. C. Fuller for permission to use the liquid scintillation counter in the Department of Microbiology at the Dartmouth Medical School during the early stages of this study.

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Effect of Gravity Compensation on the Geotropic Sensitivity of *Avena* Seedlings^{1, 2}

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Response to gravitational stimulation by geosensitive plant organs is dependent on both the magnitude and direction components of the gravity force, a vector quantity. The directional component of this vector can be nullified by clinostat rotation of the plant about a horizontal axis. Successful nullification or compensation is indicated by the growth of geotropically sensitive organs in the direction of initial orientation, with no other directional growth trends. With interest in the tropic behavior of plants developing in low g environments, we have begun studies on the effect of rotational nullification of the directional component of earth g upon tropic sensitivity. [A more explicit background and description of the various apparatus employed may be found elsewhere (9, 11)]. We have found that plants grown under these conditions of rotation differ in a number of respects from those grown under

normal orientation. This paper describes changes in geotropic sensitivity and growth of *Avena* seedlings grown under continuous nullification by clinostat rotation.

Materials and Methods

Hulled oat seeds (*Avena sativa* cv. Victory, Svalof, Sweden) were planted near the center and just below the surface of a 2-cm thick layer of moist quartz sand (ca. 15% H₂O by wt). Two hundred and fifty ml beakers were used as the experimental container; 4 seeds were planted in each. Prior to planting, seeds were soaked for 2 hours and rinsed for one-half hour in tap water at about 21°. Seeds were so oriented that the embryonic coleoptiles were pointed toward the sand surface with their longer axes parallel (fig 1a). To decrease water loss, the beakers were covered with transparent plastic film.

Immediately following planting, plants were subjected to their prescribed experimental treatment. Three distinct treatments were employed: compensation, rotation, and stationary. Compensation for

¹ Received March 22, 1965.

² Work performed under the auspices of the National Aeronautics and Space Administration.