THE EFFECT OF GRAVITY AND CENTRIFUGAL FORCES ON THE MOVEMENT OF AUXINS IN COLEOPTILE SECTIONS OF *ZEA MAYS* L.

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Rajnikorn Ouitrakul
1969

ABSTRACT

THE EFFECT OF GRAVITY AND CENTRIFUGAL FORCES ON THE MOVEMENT OF AUXINS IN COLEOPTILE SECTIONS OF *ZEA MAYS* L.

By

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Several effects of gravity on auxin movement have been documented: a lateral downward displacement when the coleoptile is placed horizontally and an inhibition of basipetal transport after inversion of the tissue. The purpose of this work was to investigate in corn coleoptiles the magnitude and time course of effects of gravity (1 x g) on axial and lateral transport of 3-indoleacetic acid (IAA) and 1-napthalene acetic acid (NAA) by comparing the transport in and against the direction of the gravitational force. A study of the effects on axial auxin transport of centrifugal forces higher than 1 x g also seemed to be useful, since such experiments might help to approach the transduction mechanism of geotropism.

The influence of gravity on axial transport was shown to be a quick effect. Inversion decreased the rate of IAA-^{14}C basipetal transport by 10-20 percent and the rate of NAA-^{14}C basipetal transport by 30-50 percent. The inhibitory effect was significant within 30 min after

inversion. The effect was constant at least over the subsequent 3 hours. NAA-^{14}C transport increased after returning the section to an upright position and the higher steady state was reestablished quickly (within 20 min).

The decrease in basipetal transport rate upon inversion under gravitational stimulus was manifested together with a decrease in velocity of the NAA-^{14}C front. The uptake of IAA-^{14}C or NAA-^{14}C was also inhibited, but to a lesser extent than transport.

Centrifugal forces of 5 x g and 10 x g in the direction of transport increased the rate of basipetal NAA-^{14}C exit over the 1 x g control. Testing transport against the direction of the applied forces, 10 x g decreased the transport rate more than 1 x g.

Acropetal movement of IAA-^{14}C in the direction of the applied force (1 x g and 5 x g) seems to be higher than the movement against the force. The rate of acropetal auxin exit, however, was very low and highly variable.

The geostimulation of lateral NAA-^{14}C transport was established within 10 min of horizontal stimulation. The lateral NAA-^{14}C transport in the physically downward direction was significantly higher than the upward movement.

An oscillation of IAA-^{14}C transport was observed, with a period length of more than 60 min.

It can be concluded that auxin movement is higher in

the direction of gravitational and/or centrifugal force than it is in the direction against the force, and that the gravity effects of these forces are expressed quickly. Centrifugal forces of 5 x g or 10 x g provide more effective stimuli than does gravity. Basipetal auxin transport, of NAA at least, is not at a maximum rate under the normal gravitational field. These results suggest that the geosensor may act by a "pressure" mechanism.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology
1969

G56212
6-25-69

ACKNOWLEDGEMENT

I wish to thank Dr. Rainer Hertel, my major professor, for his guidance and encouragement throughout the course of this work. Special thanks are also due to Dr. Barbara Filner for valuable discussion and criticisms. The interest, and encouragement of my academic advisor, Dr. Hans J. Kende, and my guidance committee Drs. Anton Lang, Robert S. Bandurski and Joseph E. Varner are greatly appreciated.

I further thank Dr. Aleksander Kivilaan for the translation of some important papers and his interest in this work. The excellent technical assistance of Miss Vicky L. Fan is acknowledged with pleasure.

This work was supported by U. S. Atomic Energy Commission Contact No. AT (11-1)-1338 and by a UNESCO fellowship (U. N. Development Programme-Thailand).

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INTRODUCTION AND LITERATURE REVIEW

a. Auxin Transport.

The movement of auxin through coleoptile sections was first studied by Went (1928) and Van Der Weij (1932, 1934), using 'natural' diffusible auxin obtained from coleoptile tips. The auxin in an agar block (donor) was applied to one end of the section and the reappearance of the growth substance in a second agar block (receptor) at the other end of the section was assayed with the *Avena* curvature test. Since the work of Goldsmith and Thimann (1962), radioactive indoleacetic acid (IAA) has been used in most cases, allowing easier and more sensitive assays. No difference has been found in the transport properties of 'natural' auxin and the synthetic IAA, and at least the diffusible auxin was demonstrated to be most likely IAA (Raadts and Söding, 1957).

As shown by Van Der Weij (1932, 1934) and more rigorously by Goldsmith (1966, 1967) auxin transport is polar, proceeding preferentially from tip to base (basipetal); auxin can move both by *active* transport and by diffusion (readily seen in anaerobic conditions). The active transport is faster than diffusion, it can proceed against a concentration gradient and it is dependent on metabolic energy. The movement of auxin is a complex process involving uptake, transport, immobilization and destruction (Goldsmith and Thimann, 1962; Goldsmith, 1968). Auxin moves through parenchymatous tissue (Hertel, 1962). Plasmatic continuity is not required. Some evidence suggested that the transport is repeated from cell to cell (Leopold and Hall, 1966; Leopold and De La **Fuente**, 1967). Electrical coupling between adjacent Avena coleoptile parenchyma cells was not detected and the resistivity of plasmodesmata appeared to exceed at least 50 times the usual resistivity of the cytoplasm. This suggests that the unimpeded movement of the molecular or ionic form of auxin via plasmodesmata is unlikely (Goldsmith, 1968). Experiments on saturating donor concentrations and with inhibitors of transport provided evidence that the sensitive and rate limiting step is the exit rather than the uptake of auxin, presumably at the plasmalemma of each cell (Hertel and Leopold, 1963b).

Auxin seems to have no covalent interaction in the transport process. Experiments with NAA-^{18}O transport by Hertel and Flory (1968) showed that no ^{18}O was lost during transport. IAA and NAA reappeared as free, unchanged molecules in the receptors (Goldsmith and Thimann, 1962; Hertel, 1962; Veen, 1967).

b. Gravity Effect on Axial and Lateral Auxin Movement.

It has been shown for over 30 years that the movement of auxin in the coleoptile can be altered by external stimuli, e.g. light or gravity (see Went and Thimann, 1937). Under the influence of gravity there are well documented effects on both lateral displacement and axial transport of auxin. Comparing axial transport, Van Der Weij (1932) inverted Avena coleoptile sections and found about a 10 percent inhibition of IAA basipetal transport. He concluded that inversion has no large effect, if any at all. Recent experiments with radioactive auxin by Hertel (1962) indicated that there is a 10-20 percent inhibition of basipetal IAA transport in inverted 5 mm corn coleoptile sections. Pickard (1969) repeated Hertel's experiment with Avena and corn coleoptile sections and also obtained a decrease of basipetal transport, but the results were very variable. Naqvi and Gordon (1966) observed that gravity caused a slight decrease of transport velocity and transport capacity in two varieties of corn. The most clear-cut effect of inversion on transport has been shown by Little and Goldsmith (1967) with 15 mm Avena coleoptile sections. When IAA-^{14}C was transported basipetally for 8 hours, the inhibitory effect was in the range of 40-80 percent. The acropetal movement was promoted. The inhibitory effect of basipetal transport increased with time and was correlated with inhibition of growth. Although inversion decreased velocity of auxin movement it had no effect on uptake, destruction or retention of auxin in the tissue.

The influence of gravity on lateral auxin movement was first shown by Dolk (1936) in experiments with Avena and corn coleoptile sections. He demonstrated that in Avena coleoptile sections a 20 min stimulation in the horizontal position induced asymmetric auxin transport which was detected when the plants were returned to an upright position.

Early experiments with radioactive auxin, in which the source had been applied and removed before horizontal exposure, failed to detect the unequal distribution of radioactivity (Ching and Fang, 1958; Reisener 1957, 1960). However, Dolk's result were later confirmed by Gillespie and Briggs (1961) on corn coleoptile sections. Experiments with IAA-^{14}C of Gillespie and Thimann (1961) showed that IAA-^{14}C held in tissue and transported out into the basal receptors underwent asymmetric distribution after geostimulation. In coleoptile halves, the IAA-^{14}C transported out into lateral receptors was more when the lateral movement was in the direction of gravity (horizontal position for the coleoptile half) than when the lateral movement was in the normal direction (vertical position for the coleoptile half) or in the direction against gravity (horizontal position for the coleoptile half) (Gillespie and Thimann, 1963; Hertel and Leopold, 1963a).

Goldsmith and Wilkins (1964) provided further convincing evidence that the asymmetric distribution reflects lateral movement of auxin within the sections. When

IAA-^{14}C was applied asymmetrically to 15mm corn coleoptile sections, the uptake within 2 hours was the same in both horizontal and vertical sections. The distribution of radioactivity in the coleoptile half away from the source was compared. The radioactivity in the lower horizontal half was more than the activity in the vertical half. This in turn was higher than the radioactivity in the upper horizontal half. This distribution of radioactivity corresponded with the curvature developed.

These data confirmed the first statement of the Cholodney-Went Theory (see Went and Thimann, 1937) which reads: "Growth curvatures, whether induced by internal or external factors, are due to an unequal distribution of auxin between the two sides of the curving organ."

c. The Problem of Geosensors and Gravity Transduction.

Accepting the theory that asymmetric auxin distribution is caused by gravity and responsible for curvature, the question arises as to how a gravitational or centrifugal force can act on this transport process. This action has the following main characteristics (for review and discussion see Audus, 1962):

1. Some gravity induced stimulatory process must reach a threshold to elicit a response. The minimum time required to reach the threshold is called <u>presentation time</u>.

2. There is an inverse correlation between the

force (g) applied and the presentation time (t) whereby g x t = constant within a certain limit of force region and at a given temperature (see also Johnsson, 1965).

3. The sub-threshold stimuli are additive and can reach the threshold level. This implies that the stimulatory process must stop and start virtually simultanously with the application and relaxation of the stimulus to account for the rather precise summation of sub-threshold stimuli. Reversal of this process thus takes place on relaxation of the stimulus, but with a speed only a fraction of that of the forward reaction.

Two main processes can be distinguished in the overall process of stimulation:

1. The physical mechanism by which plants (cells) sense the applied force (action of the force on a 'geosensor');

2. The transduction mechanism by means of which the 'geosensor', via an unknown mechanism, acts on the cell membrane of the lower side of the cell to lead to altered biological processes which ultimately result in geotropic bending.

How do plants perceive gravitational force and translate it into biochemical processes leading to geotropic bending? A number of hypotheses have been proposed for the perception mechanism of gravity by plants (see also review Audus, 1962; Pickard and Thimann, 1966). Czapek

(1898) proposed the idea that the cell might sense the weight of its own cytoplasm. Linsbauer (1907) suggested that the cytoplasmic gel can be considered as a "hanging net". Upon reorientation, distortion could occur under its own weight to modify its biochemical and physiological behavior. Stoppel (1923) pointed out that the redistributed weight of the protoplast might alter the tension in plasmodesmata and might therefore account for graviperception. Brauner (1924) proposed that the purely physical geoelectric effect upon horizontal orientation might cause lateral transport of auxin. However, Grahm (1964) had later found that the development of the geoelectric effect might be a consequence and not the cause of the asymmetric auxin distribution.

Particular attention has been received by the Statolith Theory (Haberlandt, 1900; Nemec, 1900). This theory proposes that plants perceive geostimulation by means of movement and/or pressure of one or more of their component parts (the statoliths, most likely amyloplast starch grains) that differ in density from their cellular surroundings. The primary action of statoliths could consist in mechanical pressure exerted on contact with the plasmalemma to initiate the transduction mechanism. This process may lead to the localized alteration of the membrane which in turn might result in enhancement of auxin exit in each cell. This finally produces a flux of auxin toward the lower side of the organ (Hertel and Leopold, 1963b) resulting in upward bending.

Such a postulated transduction mechanism, however, has no direct support. It is unknown whether the postulated statoliths exert a physical action via contact pressure or via another, unknown mechanism.

Considering diffusion rates, cyclosis, mobility of cell organelles and the sedimentation rate in a gravitational field it is obvious that a significant "direct" sedimentation of auxin molecules is impossible, and for fast geotropic reactions it is plausible that the amyloplasts are the geosensors, if the latter consist of moveable particles (calculation and discussion see Audus, 1962). The Starch Statolith Hypothesis has been supported by much correlative evidence (see review by Wilkins, 1966). Hawker (1932), comparing different species could show that the rate of fall of starch grains to the physically lower side showed a high positive correlation with the presentation time.

In experiments of Pickard and Thimann (1966) wheat coleoptiles depleted of amyloplast starch still showed geotropic curvature, but with a much longer lag period than the controls (about 3 hours). The ratio of curvature to growth rate, however, was the same. This lead to the conclusion that amyloplast starch grains do not play a critical role in the geotropic response. The hypothesis was proposed that the difference in hydrostatic pressure between the upper and lower sides of the cell might be translated and amplified to yield the georesponse. This difference, however, of about 1 dyne/cm^2 across 10 μ cells of a width of ca. 10 μ is exceedingly small when compared with the cell's turgor pressure of about 10 atmospheres which equals 10^7 dynes/cm^2.

d. Aim of the Work to be Reported.

In view of the similarity of the gravity effect on lateral and axial auxin transport, a study of the gravity effects on the axial auxin movement may be relevant to the problem of geotropism. Therefore the effects of different g forces on axial auxin transport were investigated and time course studies were carried out. A study of the effects of centrifugal forces larger than 1 x g might help to decide whether pressure or the mere asymmetric distribution of a possible sensor is important. Such experiments might lead to an approach to the transduction mechanism. This, in turn, might help to gain some understanding of the transport mechanism.

In order to establish the relative sequence of lateral transport and bending, the very early time course of lateral transport has to be analyzed. The geotropic bending is a quick response which starts in less than 15 min (Brauner and Zipperer, 1961). It has already been shown that a brief period of horizontal exposure is sufficient to cause lateral auxin asymmetry after return to the upright position (see above, Dolk, 1936; Hager, 1967). These experiments, however, do not clarify whether the transport process was rapidly altered in the early period of stimulation since the period of lateral transport extended over 90 min after horizontal exposure.

During the studies on the basipetal transport, oscillations of IAA transport with a period length of more than 60 min were observed; this phenomenon was also further investigated.

MATERIALS AND METHODS

a. Culture of Seedlings for Coleoptiles.

Corn seeds (*Zea mays* L. hybrid WF9x 38, lot 4243 from Bear Hybrid Corn Co., Decatur, Ill.) were soaked overnight in tap water and planted in a plastic box (covered, with an air inlet) on 6 layers of wet paper towels. The seedlings were grown in a darkroom 24°C, 80-90 percent relative humidity for about 5 days, in darkness interrupted with 2 hours of red light each night. Coleoptiles of 20-30 mm length were used. The sections were cut with the aid of 2 razor blades mounted at a fixed distance (5 or 2 mm) about 2 mm from the tips.

b. Auxin.

When investigating axial and lateral transport, IAA and mainly NAA were used. NAA is biologically active, is transported in an active and polar manner as IAA, and offers technical advantages over the latter, e.g. it is more stable for a long period of time. (See also Hertel and Flory, 1968).

3-Indoleacetic acid (=IAA) and 1-naphthalene acetic acid (=NAA) were used in both unlabeled and labeled form. Carboxyl-labeled IAA-^{14}C (specific activity 33 mC/mM) and carboxyl-labeled NAA-^{14}C (specific activity 50.6 mC/mM) were both purchased from Nuclear Chicago Corp.

c. Axial Transport Test.

To prepare agar blocks, Difco Bacto-agar 1.5 percent was melted and poured into steel molds on microscope slides chilled from underneath with ice, and cut flat. The blocks were 23 mm in diameter and 1 mm thick. Receptor blocks contained only agar; donor blocks were made by adding auxin to the molten agar before pouring it into molds (Fig. 1).

The original agar blocks as described were divided into smaller blocklets for each transport test, 3 blocks for 8-12 sections, 4 blocks for 4-6 sections, and 8 blocks for 1-2 sections.

After cutting, coleoptile sections were transferred to receptor blocks placed on microscope slides. Four plasticine columns slightly higher than the sections were placed at the four corners of the slide. The slide with the donor block was placed down on the plasticine until all sections were in good contact with the donor (Fig. 1). If not otherwise specified, upright coleoptile sections were pretreated with cold IAA and NAA for 60 min prior to application of the IAA-^{14}C or NAA-^{14}C donor in order to avoid the disturbance at set-up steady state. If inverted sections were to be used, they were inverted immediately after application of the donor by inverting the assemblies. The assemblies were kept in a plastic box (covered, with an air inlet) lined with 2 layers of wet paper towels. The manipulation was done under dim green light from a flourescent lamp covered with 4 layers of

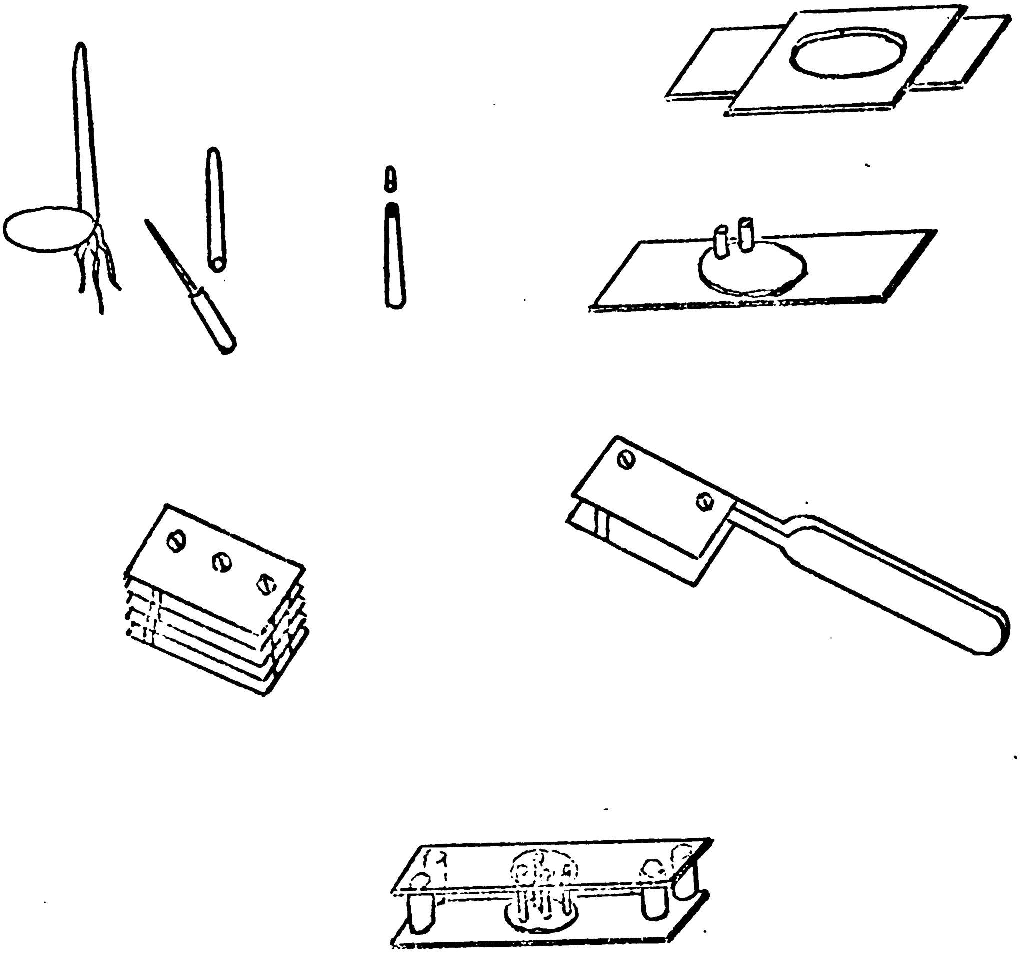


Figure 1. The auxin transport test.

yellow and blue cellophane (see Fig. 2a, for tissue orientation).

d. Lateral Transport Test.

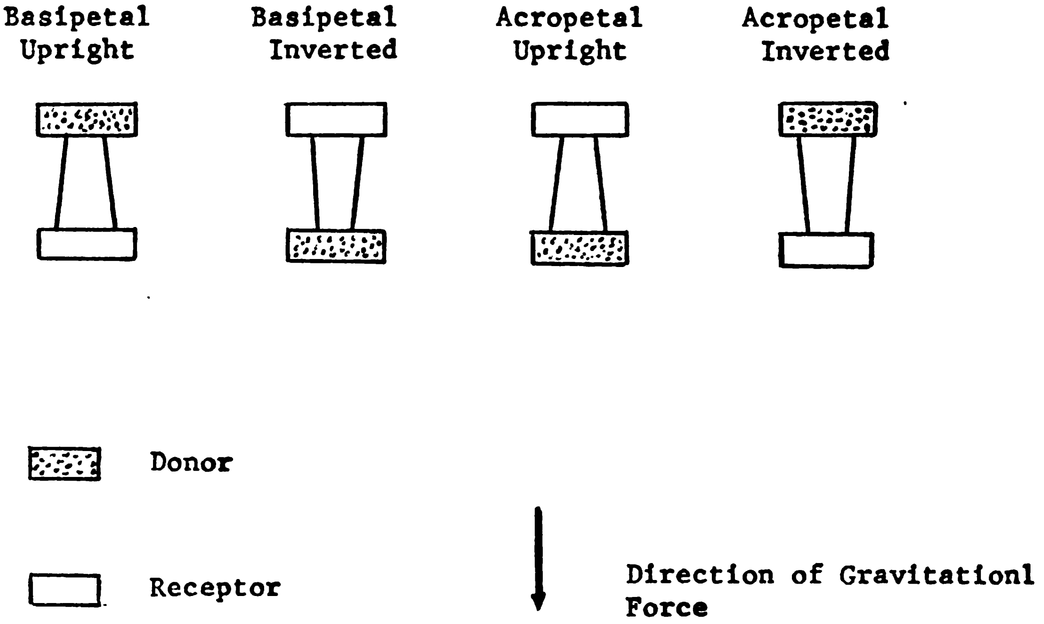
Donor blocks were 3 mm wide and ca. 15 mm long, receptor blocks were 5 mm wide and ca. 20 mm long. 5 mm long coleoptile sections cut about 2 mm below the coleoptile tips were depleted of endogenous auxin by transport between two plain agar blocks for 60 min and subsequencely were cut into halves. Each half was placed on a separate lateral receptor block so that about 2.5 mm of the tissue was on agar. The donor block was placed on the apical end of the sections. Each test was run with 5 coleoptile halves and for the same uptake and transport period. The sections in the assembly were kept in the vertical position and changed to a horizontal position for different transport intervals. Transport in the direction of gravity and against gravity was compared (Fig. 2b).

e. Orientation of Tissue Sections for the Transport Test.

To test auxin movement, auxin is applied to the tissue by a donor agar block and its reappearance is measured in the receptor block.

Axial movement is studied when the donor-receptor connection, i.e. the movement, is along the long axis of the coleoptile. Axial movement is basipetal when the donor is applied at the morphologically apical cut end of a section; the movement is towards the basal end (Fig. 2a).

a. Axial Transport Test.



b. Lateral Transport Test

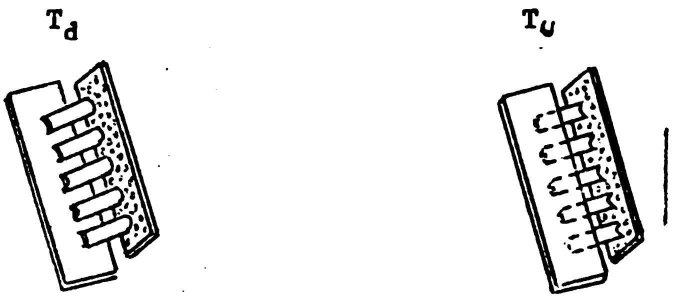


Figure 2. Orientation of tissue sections for the transport test.

Acropetal movement is in the opposite direction.

The sections can be oriented morphologically upright or inverted. In the case of basipetal movement (no centrifugal force involved) the transport in upright sections proceeds in the direction of gravity while in inverted sections it proceeds against the direction of gravity. The reverse is true for acropetal movement (Fig. 2a).

Lateral movement is assayed with sections that are sliced in half lengthwise, the donor is applied at the apical end of the half-section as in basipetal transport, but in this case the auxin allowed to exit from the longitudinal cut surface into a lateral receptor (Fig. 2b).

When centrifugal forces are applied, the movement in the direction of centrifugal force is tested when the cut surface touching the receptor faces away from the centrifuge center.

f. Special Coleoptile Centrifuge.

For experiments with centrifugal forces, a special coleoptile centrifuge, designed by R. Hertel and R. Geyer, (see Fig. 3) was used.

The centrifuge axis was vertical, thus centrifugal forces were applied in the horizontal direction.

The centrifuge arm can hold objects at the two ends with the distances from the central axis to the middle of the coleoptile boxes being 0.36m (=r). The assemblies

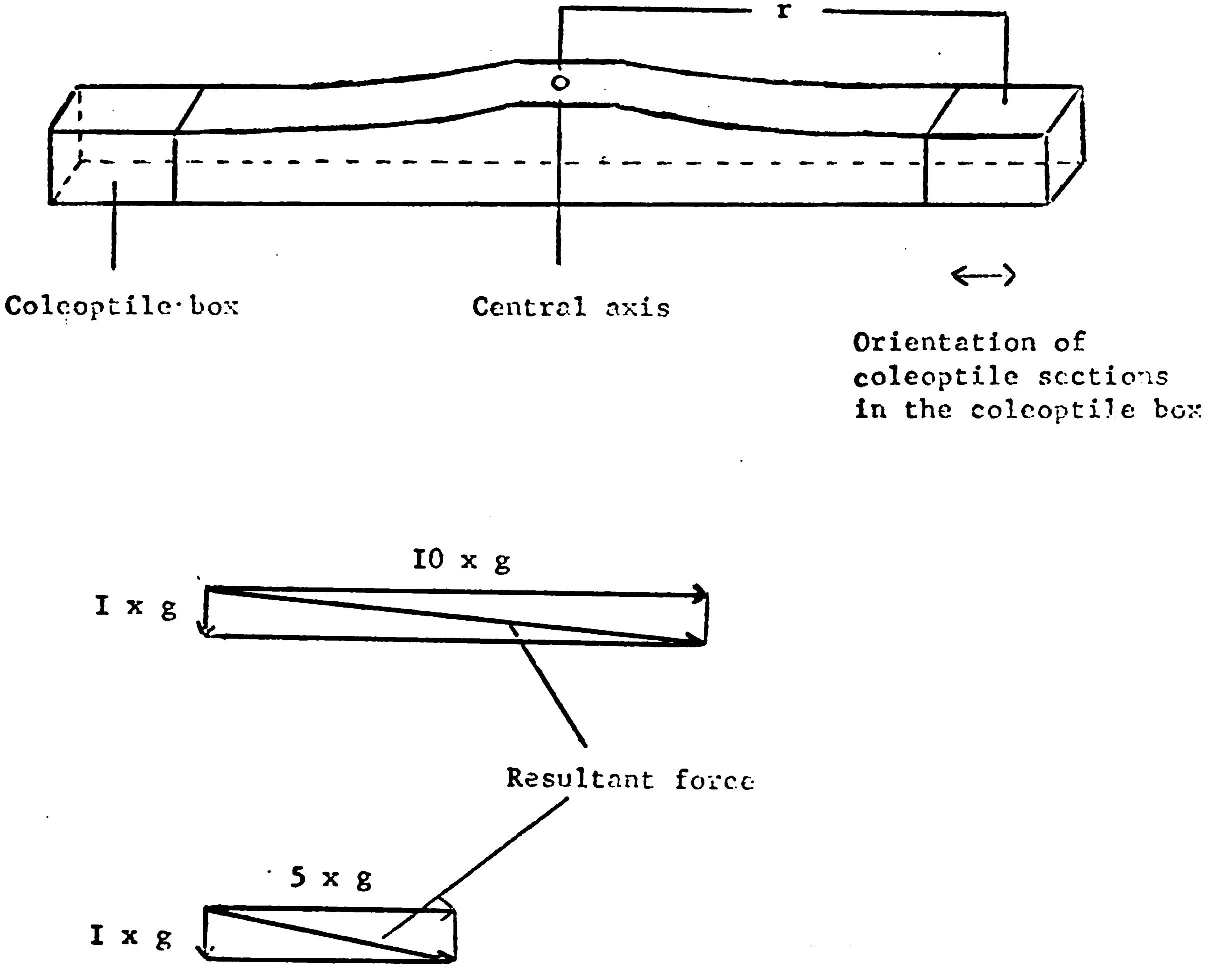


Figure 3. a. Special coleoptile centrifuge arm with coleoptile box at the two ends.

b. The vector sum (resultant force on coleoptile sections) of centrifugal force and gravitational force.

were arranged so that the coleoptiles were in a horizontal position; thus the centrifugal force was applied in the axial direction of the section. (The overall direction of the force exerted on coleoptile sections was not strictly parallel to the axis but was the vector sum of the centrifugal force and the gravitational force, see Fig. 3).

The centrifugal force is calculated from the equation

$$Z = 1.115 \times 10^{-3} \times n^2 \times r$$

where f = centrifugal acceleration (m/sec^2)

g = gravitational acceleration = 9.81 m/sec^2

$Z = \frac{f}{g}$ = centrifugal force expressed in multiples of the gravitational force g

n = revolutions per min (rpm)

if r is constant, Z is proportional to n^2

The centrifuge forces (Z) used in these experiments were 5 x g (at n = 111.6 rpm), 6 x g (at n = 122.3 rpm) and 10 x g (at n = 157.8 rpm).

Since the centrifuge had a very strong drive (3/4 horse power) the arm could be accelerated to the required g-force within seconds. Braking was achieved within 30 seconds, with the help of manually applied pressure.

g. Assay of Radioactivity.

After the end of a transport period, agar receptors and tissue were transferred to counting vials containing 5 ml of Bray's solution (4g PPO and 0.2g POPOP, both from

Packard Instr. Co., Downers Grove, Ill.; 60g naphtalene, 100 ml methanol, diluted to 1000 ml with p-dioxane) and were subsequencely allowed to extract for at least 15 min before counting at room temperature. A Beckman liquid scintillation counter Model CPM-100 (with 90 percent ^{14}C counting efficiency with window fully opened) was used. The values (cpm) given in the results section are corrected for background, which was at ca. 30 cpm in all experiments.

RESULTS

1. Effect of Gravity and Centrifugal Force on Axial Auxin Transport.

a. Basipetal Transport of IAA in Upright and Inverted Coleoptile Sections.

The effect of gravity on axial auxin movement has been documented by comparing basipetal IAA transport in upright and inverted tissue (see Introduction). To confirm this basic finding, such tests were performed using donors containing 2 x 10^{-6}M IAA-^{14}C and 2 mm long coleoptile sections. For each transport test, sections were cut from a single coleoptile (4 sections) from about 2-10 mm below the tip. The donors and receptors were changed every 30 min throughout the 4 hours of the experimental period. The results are shown in Table 1.

In 4 tests, the transport in inverted sections was inhibited on the average by ca. 30 percent in the first 30 min; after this initial period the inhibitory effect seems to be constant in the range of 10-20 percent (Table 1, also Fig. 4a, b). The radioactivity in the tissue sections was higher in the inverted sections than the upright sections, but the total uptake (=cpm in receptor + cpm held in tissue) might have been slightly less at the end of the 4 hours transporting period.

Because the counts were rather variable, the inhibitory effect in the first 30 min of transport was evaluated statistically by the Student 't' Test (Hodges and Lehmann, 1964) from the equation:

$$Z = \frac{\bar{U}-\bar{I}}{\sigma}$$

where U = cpm transported from the upright sections;

$\bar{U}$ = average of the U replicates = 119 cpm.

I = cpm transported from the inverted sections;

$\bar{I}$ = average of the I replicates = 88.5 cpm.

σ = Standard error of the mean of the difference of each replicate = 11.27 cpm.

$$Z = \frac{119-88.5}{11.27} = 2.70 > 2.58$$

The Z value shows that the difference between U and I is significant at the 1 percent level. Therefore, the effect of inversion on basipetal IAA transport was manifested at 30 min after reorientation. Differences appearing within 15 min seen in some other experiments were suggestive but not significant.

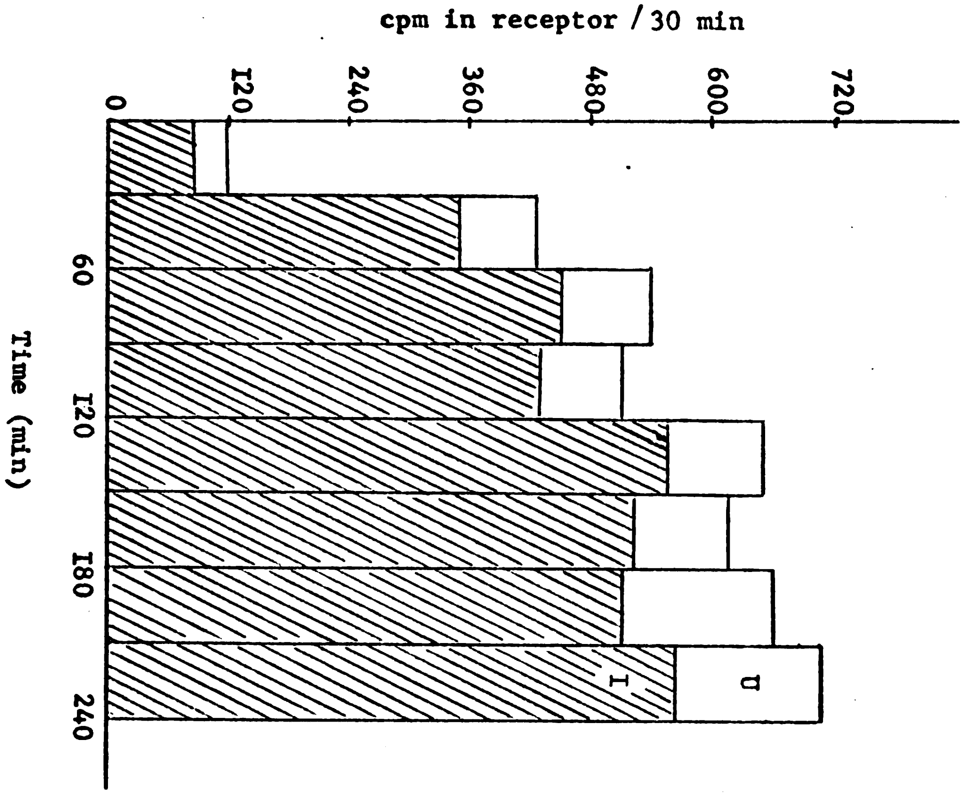
Table 1. Basipetal IAA transport in upright and inverted sections (2 mm long)

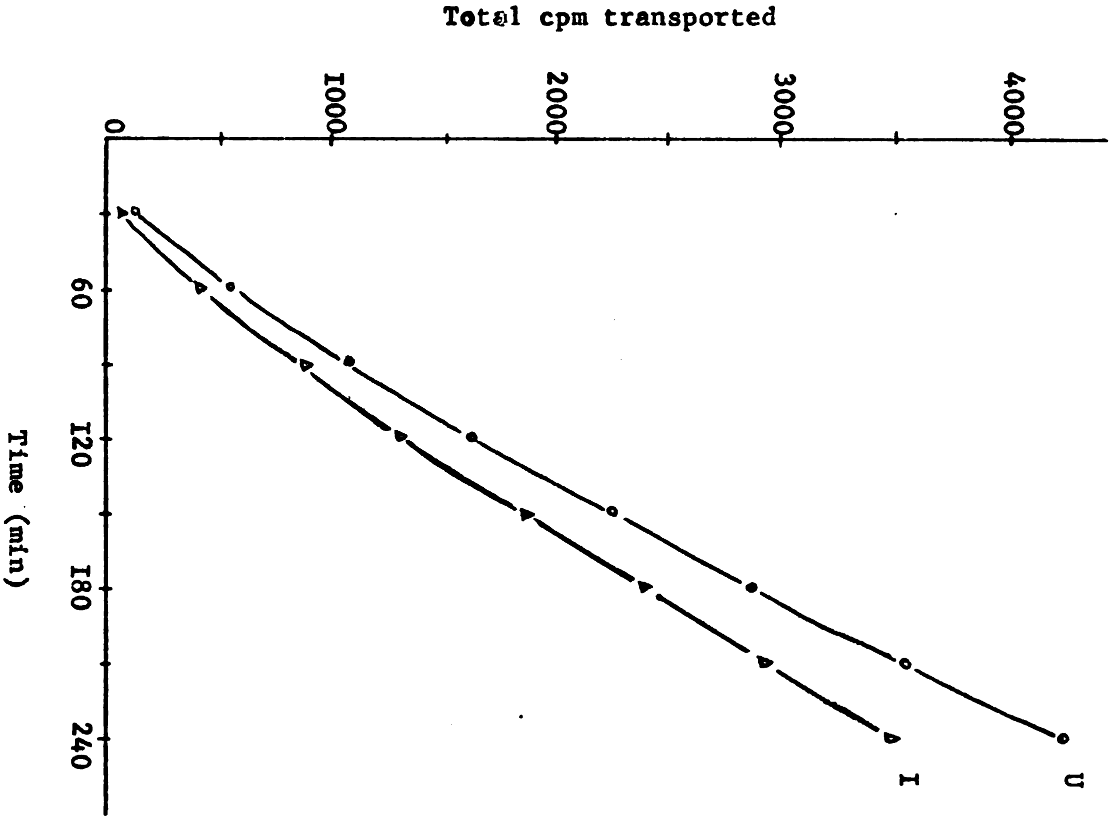
	cpm in receptors								Sum of cpm in receptors	cpm remaining in tissue	cpm total uptake
Time in minutes	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240			
Upright	151	525	611	587	778	752	823	933	5160	1515	6675
	77	323	452	437	484	475	453	500	3201	1327	4528
	160	578	687	593	742	703	742	811	5016	1563	6579
	88	320	422	436	619	558	557	618	3618	1678	5296
Average	119	437	543	513	656	622	666	716	4249	1521	5770
Standard error ±	21.27	67.27	63.43	44.33	49.06	64.01	69.18	96.77	492.84	73.08	519.67
Inverted	163	455	612	571	804	675	703	760	4743	1738	6481
	105	384	442	422	490	493	496	560	3392	2019	5411
	37	269	391	358	489	439	449	471	2903	1466	4369
	49	290	363	367	472	502	406	504	2953	1626	4579
Average	89	350	445	430	564	527	514	574	3498	1712	5210
Standard error ±	28.92	43.15	55.95	49.24	80.19	51.18	65.79	64.74	429.37	116.49	479.69
% inhibition in inverted sections	25.63	19.93	18.09	16.32	14.03	18.77	22.93	19.81	17.68	-12.56	9.70

Figure 4. Basipetal transport of IAA-^{14}C in upright and inverted coleoptile sections (data from Table 1)

a. The amount of radioactivity delivered every 30 min of transport (Table 1)

b. The total radioactivity transported until the time indicated on the abscissa.


cpm in receptor / 30 min
0
120
240
360
480
600
720
Time (min)
60
120
180
240
U
I


Total cpm transported
0
1000
2000
3000
4000
Time (min)
60
120
180
240
U
I

b. Basipetal NAA Transport in Upright and Inverted Sections.

Since experiments with IAA such as in Table 1 showed only a relatively small decrease in basipetal transport, further tests were performed with NAA transport using longer sections (5 mm). By this, it was hoped to obtain a more pronounced inhibitory effect. Coleoptile sections were cut about 2 mm below the tip, taking one section from each coleoptile. Each transport assembly contained 6 sections. Donors containing 10^{-6}M NAA-^{14}C and receptors were changed every 30 min. The results are shown in Table 2.

As in the case of IAA and 2 mm long sections, basipetal transport in inverted sections was significantly inhibited as compared with the transport in the upright ones. In the first 30 min after application of the NAA-^{14}C donor and inversion, there is almost no significant export of radioactivity (3± 1.3 cpm) whereas there were detectable counts in receptors from upright sections (16± 5.5 cpm). After this period a highly significant inhibition of 30-50 percent remained constant for a period of 4 hours. The total cpm held in tissue was more for the inverted sections but the total uptake (=cpm exported + cpm held in tissue) seemed to be less than in the upright sections.

The inversion effects on IAA and on NAA basipetal transport were compared in parallel experiments using sections of the same length. Table 3 shows the basipetal transport in 5-mm sections which were transporting 2 x 10^{-6}M IAA-^{14}C and NAA-^{14}C, respectively, for a period of 90 min. The receptors were changed every 30 min. It can be seen that the inhibitory effect on IAA-^{14}C transport was in the range of 10-30 percent whereas the inhibitory effect of NAA-^{14}C transport was about 30-50 percent as in Table 2. Thus, the amount of inhibition was consistantly higher with NAA in all experiments.

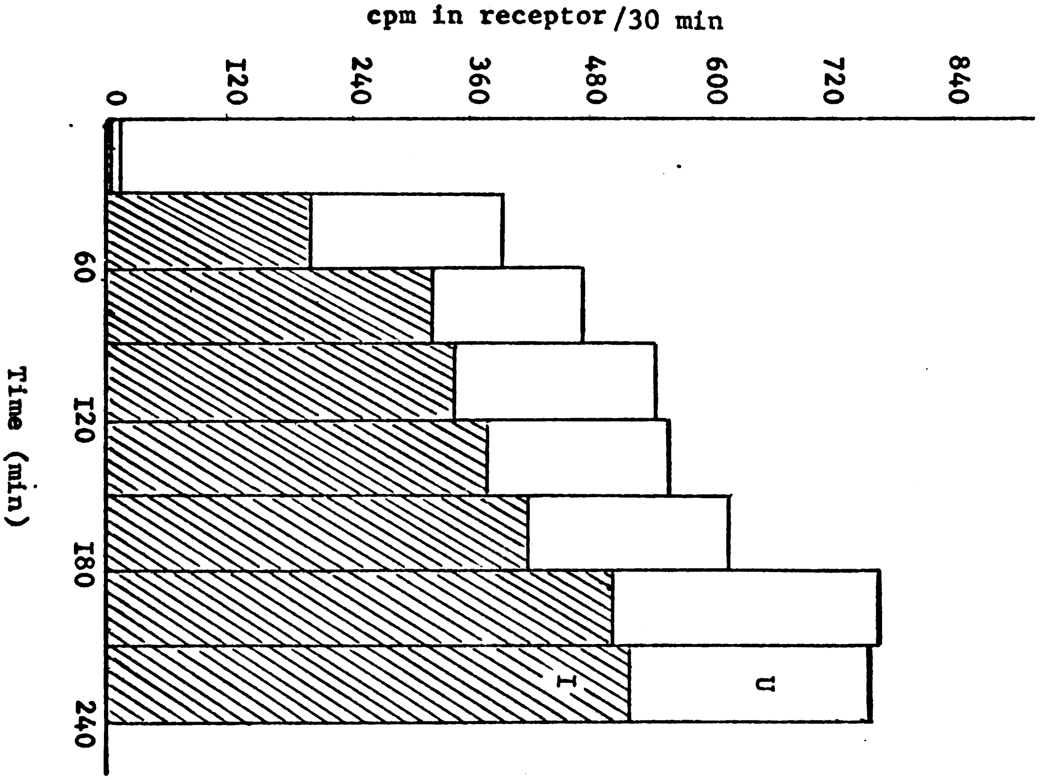
Table 2. Basipetal NAA transport in upright and inverted sections (5 mm long)

	cpm in receptors								Sum of cpm in receptors	cpm remaining in tissue	cpm total uptake
Time in minutes	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240			
Upright	24	396	466	529	580	693	815	846	4349	5772	10121
	27	423	461	567	583	600	847	848	4356	5524	9880
	6	384	507	570	532	614	699	695	4007	5392	9399
	7	370	468	520	539	566	722	651	3848	5447	9290
Average	16	393	476	547	559	618	770	760	4140	5534	9673
Stardard error ±	5.57	11.25	10.61	12.85	13.37	26.88	35.71	51.03	127.88	83.90	196.92
Inverted	6	257	379	408	401	471	589	626	3137	6893	10030
	5	168	251	248	292	348	442	447	2201	5997	8198
	1	175	326	382	363	441	523	553	2764	6747	9511
	1	209	339	340	455	385	455	450	2634	6418	9052
Average	3	202	324	345	378	411	502	519	2684	6514	9198
Stardard error ±	1.32	20.33	26.74	35.09	34.25	27.61	48.18	43.35	193.09	199.00	388.53
% inhibition in inverted sections	81.25	48.60	31.93	36.92	32.40	33.50	34.89	31.71	35.17	-17.71	4.91

Figure 5. Basipetal transport of NAA-^{14}C in upright and inverted coleoptile sections (data from Table 2)

a. The amount of radioactivity delivered for every 30 min of transport (Table 2)

b. The total radioactivity transported until the time indicated in the abscissa.


cpm in receptor/30 min
0
120
240
360
480
600
720
840
Time (min)
60
120
180
240
I
U

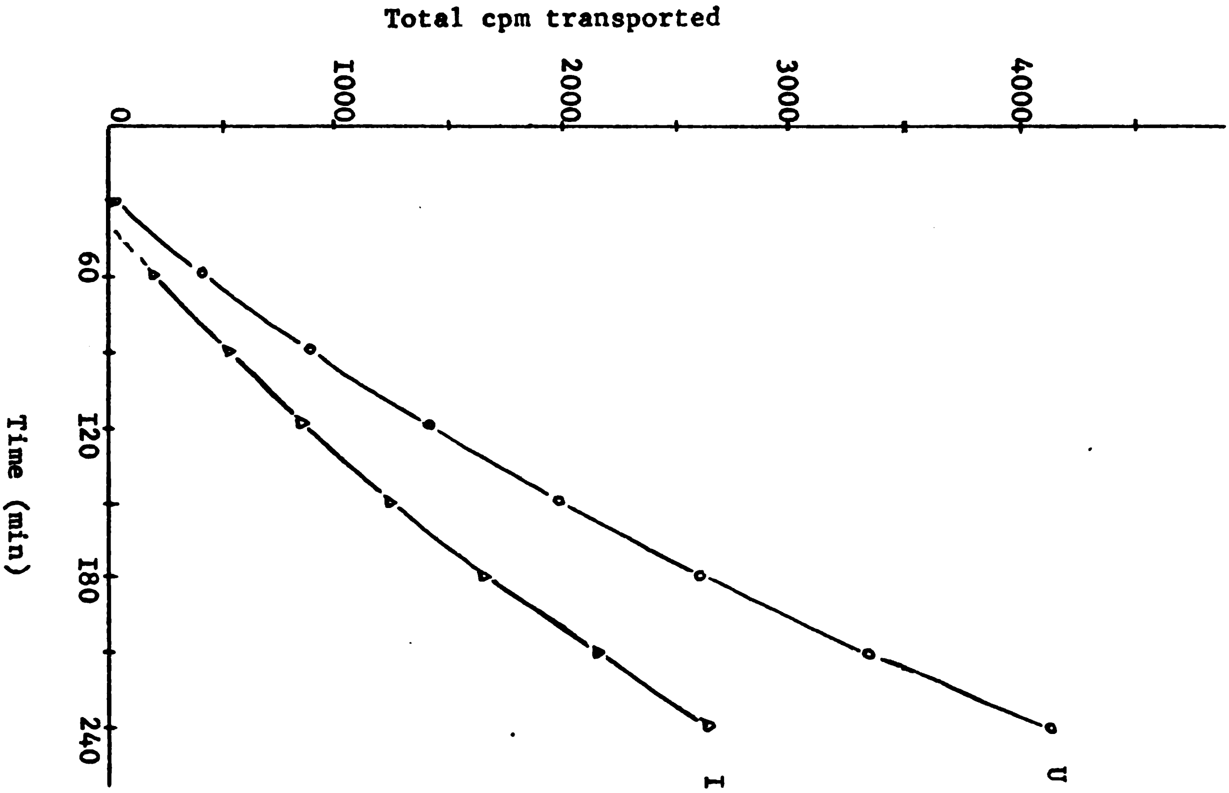

Total cpm transported
0
1000
2000
3000
4000
Time (min)
60
120
180
240
U
I

Table 3. Comparison of the gravity effects on basipetal IAA and NAA transport

		cpm in receptors			Sum of cpm in receptors	cpm remaining in tissue	cpm total uptake
Time in minutes		0-30	30-60	60-90			
Experiment I	Upright	362	822	1057	2241	1117	3358
		297	761	996	2054	1033	3087
IAA		330	792	1027	2149	1075	3224
	Inverted	247	727	910	1884	1157	3041
		333	779	910	2052	1205	3257
		290	753	925	1968	1187	3149
% inhibition		12.12	4.92	9.93	8.42	-9.86	2.32
	Upright	111	700	750	1561	2476	4037
		99	770	880	1749	2687	4436
NAA		105	735	815	1655	2582	4236
	Inverted	56	487	525	1068	2839	3907
		56	547	452	1055	2543	3598
		56	517	489	1062	2691	3753
% inhibition		46.67	29.66	40.00	35.83	-4.22	11.42
Experiment II	Upright	306	745	775	1826	932	2758
		274	680	713	1667	1038	2705
IAA		290	713	744	1747	985	2732
	Inverted	243	626	633	1493	977	2470
		163	455	618	1236	1064	2300
		199	501	626	1365	1021	2385
% inhibition		31.38	29.73	15.86	21.87	-3.65	12.70
	Upright	64	543	658	1265	3353	4618
		56	533	632	1221	3422	4643
NAA		60	538	645	1243	3386	4631
	Inverted	23	219	310	552	3987	4539
		27	234	358	619	3184	3803
		25	227	334	586	3586	4171
% inhibition		58.33	57.81	48.86	52.86	-5.91	9.93

c. Auxin Transport Rate in Sections After Return From Inverted to Upright Position.

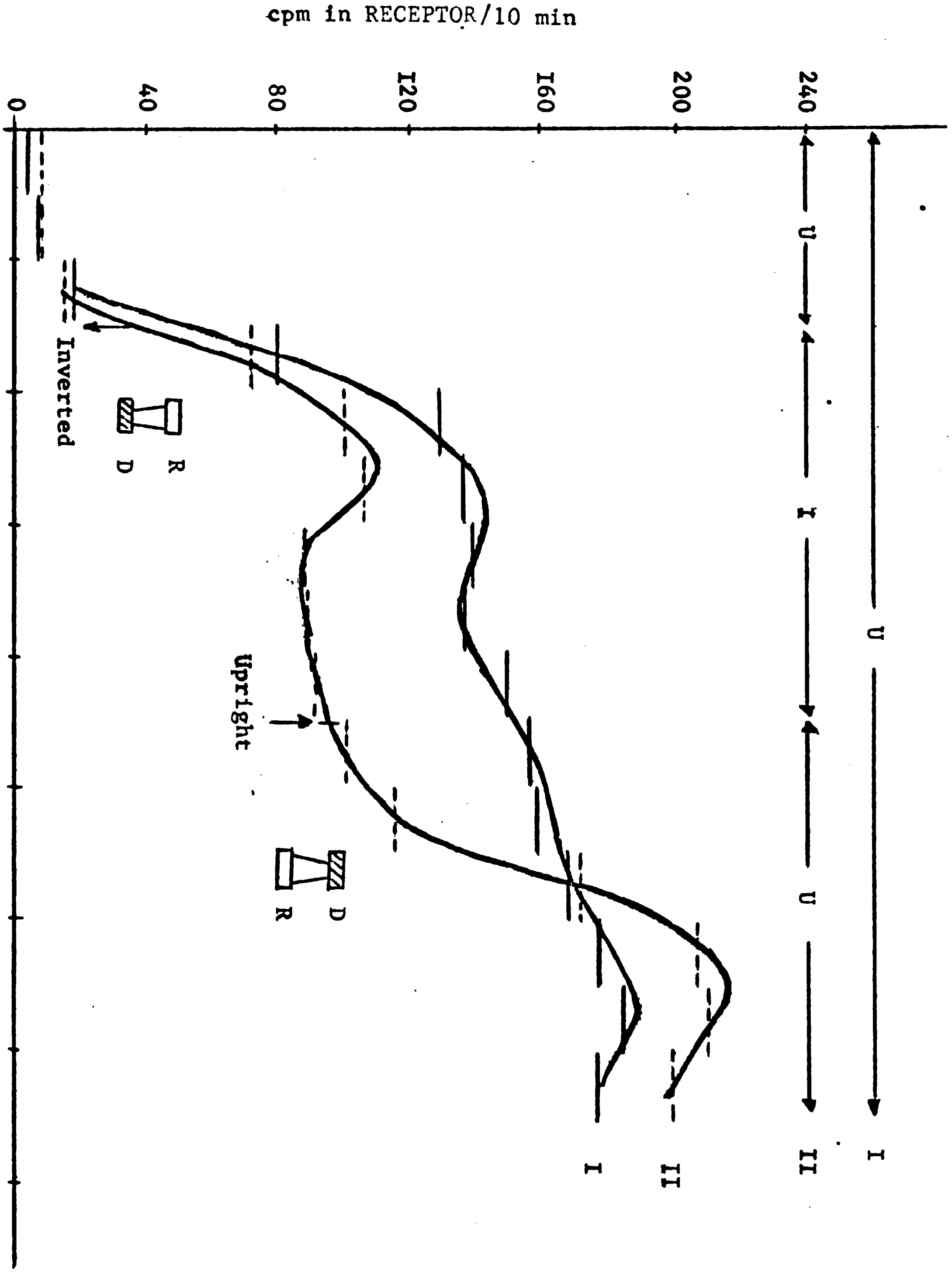
In order to test decrease of the transport by inversion and to further examine the time course of the gravity response, inverted coleoptile sections when transporting NAA at a steady rate were returned to an upright position. 5 mm coleoptile sections were pretreated with 10^{-6}M unlabeled NAA for 60 min. Four sections for each transport test were used. 10^{-6}M NAA-^{14}C was applied in the donors and the receptors were changed and collected every 10 min. The tissue sections were allowed to transport NAA-^{14}C for 30 min in the upright position; then they were inverted and NAA-^{14}C movement continued in this position for 60 min. After this period the tissue sections were erected and allowed to transport for another 60 min. The control assemblies transported in the upright position throughout the transport period.

Table 4 and Figure 6 show that the decrease in transport rate of the inverted sections appeared very early, perhaps within 10 min. After 30 min the transport rate of the inverted sections seems to reach the low steady state compared to controls. Reorientation of tissue sections to the upright position increased the transport rate observably after 10 min. After 20-30 min in the upright position the transport rate seemed to have returned to the control level, perhaps with an overshoot.

Table 4. Auxin Transport Rate in Sections After Return From Inverted to Upright Position

	cpm in receptors															
Time in minutes	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120	120-130	130-140	140-150	cpm in tissue
Control (Upright thoughout)	6	7	13	67	121	135	142	130	139	146	151	158	164	176	177	3018
	2	6	22	93	136	139	137	144	160	168	166	179	194	198	176	3072
	4	7	18	80	129	137	140	137	150	157	159	169	179	187	177	3045
Standard error ±	2.0	0.7	4.5	13.0	7.5	2.0	2.5	7.0	10.5	11.0	7.5	10.5	15.0	11.0	0.7	27.0
Treatment	10	8	16	75	107	113	94	101	110	113	120	180	207	204	213	3022
	6	7	13	69	93	98	83	79	73	91	112	166	207	218	187	2901
	8	8	15	72	100	106	89	90	92	102	116	173	207	211	200	2962
Standard error ±	2.0	0.7	1.6	3.0	7.0	7.5	5.5	11.0	18.5	11.0	4.0	7.0	0	7.0	13.0	60.5
	← U →			← I →						← U →						

Figure 6. Auxin transport rate in sections after return from inverted to upright position (data from Table 4).


cpm in RECEPTOR/10 min
0
40
80
120
160
200
240
TIME (min)
20
40
60
80
100
120
140
160
Inverted
R
D
Upright
D
R
U
I
U
I
II
I
II

d. Effect of Centrifugal Force on Basipetal NAA transport.

After an effect of gravity (1 x g) on basipetal auxin transport in upright and inverted sections had been shown above, further experiments were done to investigate the basipetal transport in or against the direction of centrifugal forces higher than 1 x g.

Table 5 shows the basipetal transport along the direction of different g forces. The experiments were done with 5 mm sections; 6 sections for each transport test were used. The tissue was depleted for 60 min prior to the application of a 10^{-6}M NAA-^{14}C donor. The ratio of radioactivity in the receptor over the total uptake (= cpm in tissue + cpm in receptor) was calculated and taken as a corrected measure of transport.

As shown in Table 5a, after 60 min of transport, basipetal auxin movement appeared to be increased in the direction of higher centrifugal force when compared to the 1 x g control.

With the technique described artifacts may be possible; e.g. little pieces of the donor or water droplets containing NAA-^{14}C could be driven towards the receptor when transport was in the direction of high centrifugal forces. To eliminate such possibilities and to further test the effect of centrifugal force, the pulse technique of Goldsmith (1967) was used. A donor with 10^{-6}M NAA-^{14}C was applied for 30 min at 1 x g to all sections; it was then changed to plain agar (Table 5b). The NAA-^{14}C within the sections was allowed to be transported basipetally for a further 30 min at 1 x g, 5 x g and 10 x g. The results show that the ratio of average cpm transported over uptake is more at 5 x g and 10 x g than at 1 x g. Thus the basipetal transport is indeed increased over the 1 x g control. The increase in basipetal transport along the direction of centrifugal force, then, is a genuine effect.

The pulse technique of Goldsmith (1967) was also used to verify whether an increased force against the direction of transport will result in a greater inhibitory effect than 1 x g. Two experiments were performed in which the tissue sections were pretreated with unlabeled 2 x 10^{-6}M IAA and 10^{-6}M NAA, respectively. A donor with 10^{-6}M NAA was applied for 30 min at 1 x g for all of the assemblies. After this uptake period the sections were transferred to plain agar receptors and donors and allowed to transport in the direction against the force in 10 x g and 1 x g. A set of coleoptiles for transport in the direction of force at 1 x g served as the control. The ratios of radioactivity exported into the receptors over total uptake were compared. It can be seen from Table 6 that the transport against the direction of force was inhibited and this inhibitory effect increased with an increase in the force applied.

Table 5. Basipetal NAA transport in the direction of gravity and centrifugal forces.

a. Donor present during transport period.

b. Movement of NAA-^{14}C pulse; all donors were present for 30 minutes at 1 x g before the centrifugal treatment was started.

	Transport under various centrifugal forces				Transport under gravity (1 x g) as control			% increase under centrifugal forces
	Forces	Receptor	Tissue	Receptor / Total Uptake	Receptor	Tissue	Receptor / Total Uptake	
		cpm			cpm			
a.	2 x g	683	1593	0.300	581	1330	0.304	-1.32
	6 x g	530	1408	0.273	330	1239	0.210	30.00
		572	1407	0.289	519	1300	0.285	1.40
	10 x g	102	3012	0.033	52	3013	0.017	94.12
		892	1834	0.327	602	2071	0.225	45.33
b.	5 x g	607	1029	0.371	461	1076	0.300	23.67
		657	1179	0.358	586	1182	0.331	8.16
	10 x g	454	867	0.344	317	761	0.294	17.00
		600	675	0.471	603	879	0.407	15.72
		445	466	0.488	386	448	0.463	5.40
		668	978	0.406	472	1140	0.293	38.57
		623	875	0.416	453	1007	0.310	34.19

Table 6. Basipetal NAA transport against the direction of gravity and centrifugal forces.

a. Pretreatment with IAA.

b. Pretreatment with NAA.

	Transport against centrifugal forces (10 x g)			Transport against gravity (1 x g)			Transport in the direction of gravity (1 x g) (control)			% inhibition with gravity	
	Receptor	Tissue	$\frac{\text{Receptor}}{\text{Total Uptake}}$	Receptor	Tissue	$\frac{\text{Receptor}}{\text{Total Uptake}}$	Receptor	Tissue	$\frac{\text{Receptor}}{\text{Total Uptake}}$	10 x g	1 x g
	cpm			cpm			cpm				
a.	628	957	0.396	778	997	0.438	644	905	0.416		
	532	1166	0.313	757	932	0.448	837	865	0.492		
	580	1062	0.354	768	965	0.443	741	885	0.454	22.03	2.42
b.	366	1094	0.251	558	977	0.363	589	890	0.398		
	455	1182	0.278	484	894	0.351	669	860	0.438		
	411	1138	0.264	521	936	0.035	629	875	0.418	36.84	14.59

e. Effect of Gravity and Centrifugal Force on Acropetal Movement.

From the results on the basipetal auxin transport, one might expect that acropetal auxin movement is also higher when in the direction of gravitational force than when against it, regardless of the mechanism of the movement, active transport or diffusion. Although obtaining only relatively small **amounts** of acropetal auxin exit, Little and Goldsmith (1967) could verify this prediction.

In order to confirm the gravity effect on acropetal movement, the inversion effect was tested in preliminary experiments using 8 sections (2 mm long; four sections from each of 2 coleoptiles) for one transport test. The results show that there were no receptor counts significantly above the background when 10^{-6}M IAA-^{14}C was transported for up to 160 min and 10^{-6}M NAA-^{14}C up to 240 min. When the IAA-^{14}C concentration was increased to 5×10^{-6}M, a significant fraction of auxin was delivered into the apical receptors after 90 min of transport. (The absence of counts in receptors during transport of 10^{-6}M radioactive auxin transport indicates that there is no leakage of auxin via external moisture into the receptors.)

Further experiments were performed to test the effect of the direction of gravity and centrifugal force on acropetal auxin movement using relatively high donor concentrations. The radioactivity in the apical receptors of upright and inverted sections was assayed after 90 min of transport.

The data (Table 7) are highly variable, but taken together the experiments provide suggestive evidence that the acropetal movement in the inverted sections was enhanced over that in the upright sections. Also, when a 5 x g centrifugal force was applied (Table 7d) the movement in the direction of the force seemed to be higher than that against it.

Table 7. Acropetal movement at 1 x g and 5 x g of IAA-^{14}C for 90 min after 60 min pretreatment with 5 x 10^{-6}M cold IAA. All donors contained 5 x 10^{-6}M IAA-^{14}C; the final concentration was made by adding appropriate amount of unlabeled IAA. In a, b and c gravity (1 x g) was the applied force, while in d movement with and against centrifugal force (5 x g) was tested. Each transport test contained 12 sections (2 mm long) cutting from 3 coleoptiles.

Donor Concentration	Upright Receptor	Upright Tissue	Upright Total Uptake	Inverted Receptor	Inverted Tissue	Inverted Total Uptake
	cpm			cpm		
a.	278	2984	3262	38	3487	3525
	177	3313	3490	859	4415	5274
	30	3903	3933	37	3651	3688
10^{-5} M	263	3458	3721	312	3418	3730
	283	3304	3581	608	3148	3756
	582	3273	3855	45	3227	3272
Average	269±73	3373	3642	317±142	3557	3874
b.	442	1797	2239	840	2264	3104
	77	2089	2166	29	2068	2097
	76	1871	1947	19	2029	2048
1.5 x 10^{-5} M	377	2183	2560	57	1862	1919
	88	1944	2032			
	278	1783	2061	801	2263	3064
Average	223±67	1945	2168	349±193	2105	2454
c.	16	1540	1556	38	1900	1938
	38	1732	1770	180	2208	2388
	30	1944	1974	64	1883	1947
5 x 10^{-5} M	81	1588	1669	15	2276	2291
	79	2111	2190	170	1930	2100
	49	1664	1713	39	2035	2074
Average	49±11	1763	1812	84±29	2039	2123

Centrifugal Force 5 x g

	In the Direction of Force Receptor	In the Direction of Force Tissue	In the Direction of Force Total Uptake	Against the Directon of Force Receptor	Against the Directon of Force Tissue	Against the Directon of Force Total Uptake
	cpm			cpm		
d.	92	5148	5240	80	4817	4897
	21	4419	4440	180	4588	4696
5 x 10^{-5} M	20	4993	5013	31	4463	4494
	16	5261	5277	202	5205	5407
Average	37±18	4955	4992	105±36	4768	4873

2. Time Course of the Effect of Gravity on Lateral NAA Transport.

After it had been shown that both basipetal and acropetal auxin movement are affected by gravity and centrifugal forces and that this effect is a rapid one, the gravity effect on lateral auxin transport and its time course were examined since this transport is thought to play an important part in the geotropic response.

The lateral transport in the physically upward and downward direction was compared using coleoptile halves. NAA was used since its axial movement had shown more pronounced gravity effects than did the IAA transport; and since NAA can mediate a geotropic bending reaction (Anker, 1962), but its lateral displacement under the influence of gravity had not yet been documented.

A test consisted of two transport assemblies (see Material and Methods, Fig. 2b), each with 5 coleoptile halves. Donors with 10^{-5}M NAA-^{14}C were applied after 60 min of depletion. The sections in the assemblies were left in the upright position for different time periods (t_v = 0, 10, 15, 20, 30 min) and then changed to the horizontal position to allow transport in the downward (T_d) or upward (T_u) direction for t - (30-t_v) min. All counts arriving in the lateral receptor during the total 30 min were registered, including those that were exported during the upright exposure. In Fig. 7 the time of horizontal exposure (t) is shown on the abscissa. The lateral transport was evaluated in each test as the percent difference of downward minus upward transport over the sum of transport in both assemblies.

$$\text{NAA asymmetry } (\%) = \frac{T_d - T_u}{T_d + T_u} \times 100$$

The percent of NAA asymmetry was averaged from many experiments performed at different times. Its mean value and the standard error of the mean were calculated and the results are shown in Fig. 7. It can be seen that a difference could be observed within 10 min after reorienting the tissue.

Table 8. Lateral transport of 5 coleoptile halves (5 mm long) from donor containing 10^{-5}M NAA-^{14}C in the downward direction (T_d) (with gravity) and upward direction (T_u) (against gravity) after different times in horizontal position (see text, Material and Methods).

Time in Horizontal Exposure	cpm in receptor		
	T_1^*	T_2^*	$\frac{T_1 - T_2}{T_1 + T_2} \times 100$
0 min	27	31	-16**
	25	36	-18
	72	40	29
	9	7	13
Average	33	29	8±12.1

* cpm transported in vertical position

** the mean value of $T_1 - T_2$ is corrected to be zero

	T_d	T_u	$\frac{T_d - T_u}{T_d + T_u} \times 100$
10 min	17	8	36
	47	11	62
	41	29	17
	31	14	38
	29	21	16
	82	44	30
	7	9	-13
Average	36	19	27±8.8
15 min	80	36	38
	26	13	33
	32	12	45
	49	24	34
	19	7	46
Average	41	18	39±2.7
20 min	17	9	31
	46	9	67
	98	42	40
	95	20	65
	120	19	73
	55	25	38
	17	13	13
	26	9	49
	87	24	67
	15	8	30
Average	58	18	47±6.4
30 min	45	16	48
	55	22	43
	79	14	70
	82	12	74
	31	5	72
Average	58	14	61±6.6

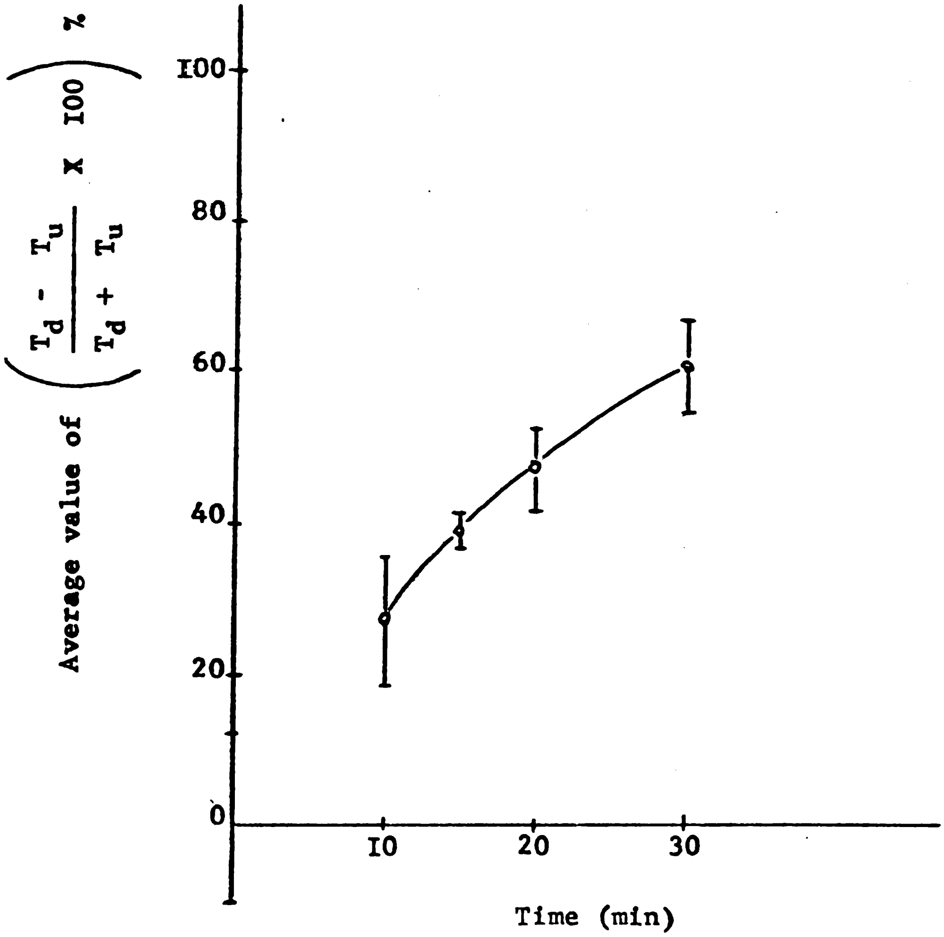


Figure 7. The percent difference in lateral transport in the downward direction along gravity as compared to the upward direction against gravity as a function of time in horizontal position (see Table 8, text and Material and Methods).

3. Oscillation of Auxin Transport.

In many experiments on basipetal IAA-^{14}C transport (e.g. Table 1) in which the receptors were changed at intervals up to 30 min, the rate of delivery of radioactivity showed a more or less pronounced oscillatory pattern with a period length of ca. 60-100 min. The data in Table 9, Fig. 8, document a particularly clear case of transport oscillation. In this experiment, the basipetal transport of 5×10^{-6}M IAA-^{14}C in a single 2 mm coleoptile section was tested. Prior to the start of the experiment the seedlings were kept in 27°C and 90 percent relative humidity for about 30 hours. The transport test, however, was performed at the standard temperature of 24°C.

After cutting, the sections were either directly (that is immediately) placed in a transport assembly, or were first subjected to a depletion period of 40 min. Donors and receptors were changed every 25 min. The transport in both series was compared. For each series, 4 sections from one coleoptile were used which all show a similar transport pattern (Table 9; example in Fig. 8a and b; in Fig. 8c, the average of 4 sections is given). In sections used immediately after cutting a minimum of transport was reached at about 140 min after cutting and donor application (e.g.,Fig. 8a and c). In tissue depleted for 40 min the minimum was reached at about 100 min (e.g., Fig. 8b and c) after donor application, that is also 140 min after cutting. This may indicate that the oscillation had been started at the moment of cutting the sections (see also Kirk and Jacobs, 1968).

Table 9. Basipetal IAA transport in single coleoptile section (2mm long)

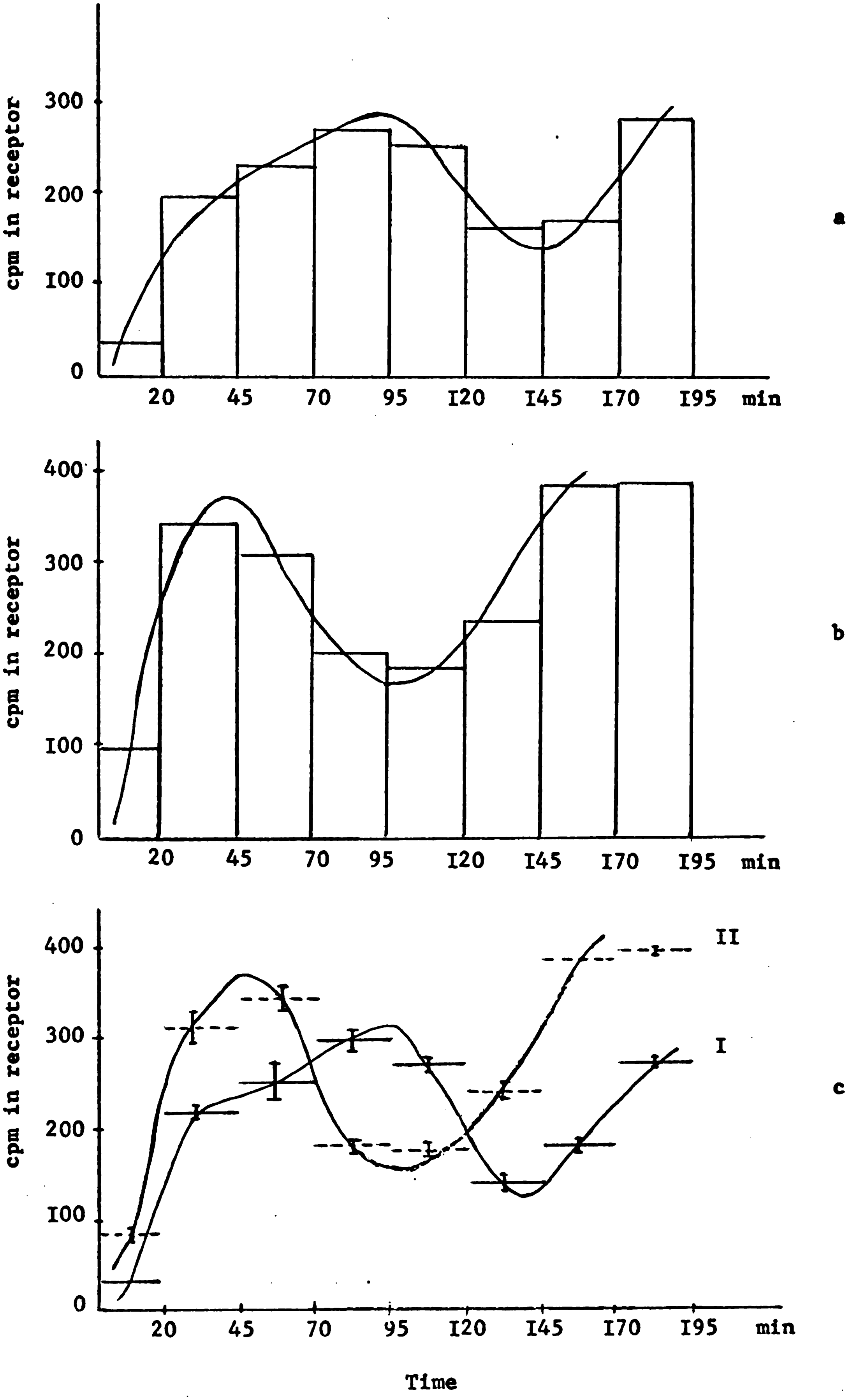
	cpm in receptor							
Time in minutes	0-20	20-45	45-70	70-95	95-120	120-145	145-170	170-195
Transport after cutting								
section No.1	27	246	325	330	280	126	201	269
2	33	196	228	270	255	164	169	285
3	35	206	218	267	271	133	172	274
4	33	217	222	309	275	136	178	257
Average	32±2	216±11	248±26	294±15	270±5	140±8	180±7	271±6
40 min. depletion								
section No.1	98	344	309	203	185	237	389	390
2	87	327	319	172	158	251	390	408
3	88	309	383	164	177	248	386	388
4	54	251	253	182	182	225	381	392
Average	82±10	308±20	341±17	180±6	176±6	240±6	387±2	395±5

Figure 8. Basipetal transport of 5×10^{-6}M IAA-^{14}C in 2 mm single coleoptile sections. The donors were applied at t = 0 in all cases. The section had been cut at t = 0 for Fig. 8a and c (curve I), but at t =-40 min for Fig. 8b and c (curve II, depleted). The transport rate referred to cpm in the receptor after time indicated in the abcissa.

a. The transport rate in section No. 2 (Table 9, after cutting)

b. The transport rate in section No. 1 (Table 9, depletion)

c. The average transport rate of 4 sections in each treatment, with standard error of the mean. Curve I like a; curve II like b (depleted).


cpm in receptor
0
100
200
300
20
45
70
95
120
145
170
195
min
a
cpm in receptor
0
100
200
300
400
20
45
70
95
120
145
170
195
min
b
cpm in receptor
0
100
200
300
400
20
45
70
95
120
145
170
195
min
II
I
c
Time

DISCUSSION

The purpose of this investigation was to describe and compare effects of gravity and centrifugal force on the auxin movement in coleoptile sections. The general conclusion can be drawn that auxin movement is higher in the direction of gravitational and/or centrifugal force than it is in the direction against the force. This holds true for axial movement - both basipetal and acropetal - as well as for lateral transport.

As discussed in the Introduction, there is substantial evidence for a gravity induced lateral downward movement of IAA. The present results show the same pattern for the auxin NAA (Fig. 7).

It was confirmed that basipetal transport of IAA is decreased when the movement has to proceed against gravity (Fig. 4). This finding is in agreement with several earlier reports (e.g. Hertel and Leopold, 1963a; Naqvi and Gordon, 1966; Little and Goldsmith, 1967). The basipetal transport of NAA was also shown to be sensitive to inversion; the difference in movement between the upright and inverted position was greater with NAA than with IAA (Table 3). An inhibition by inversion has also been reported for the movement of 2,4-D (Hertel and Flory, 1968).

The data on acropetal auxin movement show great variability. However, pooling all results (Table 7, especially the higher IAA concentrations) a significant difference can be seen: more auxin moves acropetally in the direction of the gravitational force than the opposite direction.

Concerning the inhibition of basipetal transport by inversion, the results suggest that mainly the transport or exit rate is affected by gravity. A decrease in velocity can be observed in Figure 5. The NAA front of the inverted sections seems to appear later than that of the upright ones (see also Fig. 5 in Little and Goldsmith, 1967).

Inversion has been reported to have no effect on auxin uptake (Little and Goldsmith, 1967). However, from data in Table 1, 2 and 3 a slight inhibition of uptake is detectable. This uptake inhibition increased whenever the transport inhibition increased.

When studying the early time course after a gravitational stimulus the effects both on lateral and on axial transport seem to be rapid; they are already visible ca. 10 min after the change in orientation.

Under the Cholodny-Went theory, a rapid response (15 min) of lateral auxin movement to gravity is required to account for the short latency period for geotropic bending (ca. 15 min; e.g. Brauner and Zipperer, 1961) and the geoelectric effect (ca. 15 min; Grahm and Hertz, 1962).

For NAA-^{14}C movement, it was possible to demonstrate a gravity induced lateral displacement within 10 min after reorientation (Fig. 7). With IAA-^{14}C, the increase of movements in the downard direction to a lateral recepter was observable within 15 min after geostimulation (B. Filner, personal communication).

A stimulation of basipetal NAA transport was established quickly upon returning the coleoptile sections from the inverted to the upright position (Fig. 6). A difference could be observed 10 min after orientation. At 20 min the axial transport system seemed to function at the level in the upright controls.

In the standard test of the inversion effect, a significant decrease of the exit rate of IAA and NAA was detected within 30 min (e.g. Fig. 4, 5 and 6). The relative quickness of the gravity effect on axial transport is further documented in data of Little and Goldsmith (1967, Fig. 5) where a retardation of a moving pulse is seen 30 min after inverting the sections.

In our experiments, the relative difference between the transport with and against the direction of force did not further increase with time after ca. 30 min. Little and Goldsmith (1967) reported the increase in the inhibitory effect of inversion with time. This discrepancy may be due to the longer transport period and to the higher concentration of IAA used by them.

The geostimulation of the transport system is quick; no conclusions can be drawn from the experiment reported here concerning the decay of the stimulated state. Data of Dolk (1936) and especially of Hager (1967) indicated that the capacity for geoinduced lateral transport remains for sometime after returning the tissue from a horizontal to an upright position. The stimulated state decays with a half life of ca. 30-40 min (Hager, 1967), in contrast to the rapid start of the stimulation.

Centrifugal forces over 1 x g provide more effective tropistic stimuli than gravity (1 x g). Within certain dosage limits, increasing the force will increase the resulting curvature (see in Rawitscher, 1932). From the Cholodny-Went theory one must expect that lateral auxin transport will also be increased under laterally acting, high centrifugal forces. This has been confirmed by Filner and Hertel (personal communication). In the experiments reported here it was shown that centrifugal forces acting longitudinally on the coleoptile section do have effects on the axial auxin movement.

Forces over 1 x g had a rapid effect on enhancement of basipetal transport of NAA when the movement was in the direction of force (Table 5). The basipetal transport of NAA against the force was more decreased in the 10 x g case than in the 1 x g control (Table 6). Preliminary results indicate that the acropetal movement shows a similar pattern; more IAA was transported in the direction of the force when it was 5 x g than when it was only gravity (Table 7d).

Especially interesting is the fact that the basipetal auxin transport, at least that of NAA, is not at maximum rate in the normal gravitational field.

The results reported above may be relevant to the question of whether possible statoliths - **amyloplasts** - act by pressure or by asymmetric distribution, e.g. as a source of some transport promoting chemical.

The case of the increase of basipetal transport by increased centrifugal force (Table 5) in the direction of movement can not easily be explained by mere asymmetric distribution of statoliths. In this case, the movable amyloplasts are already collected at the lower side of the cells, prior to the actual transport test although not at tightly packed as with the higher centrifugal force. Statolith particles, thus, may possibly act by exerting pressure on a sensitive structure, and/or by being pressed into the "gel" proximity of the membrane (see also Hertel, 1969).

This suggestion is based on the assumption that the geosensors are particles. The results, however, do not discriminate between a statolith hypothesis and a hypothesis that involves the pressure of the whole cell content, or a "hanging net".

From all the results reported here the effects on axial and on lateral transport appear to be very similar. This would be easily understood if it is assumed that the axial and lateral auxin transport systems are qualitatively the same. It is reasonable to visualize that the whole plasma membrane contains the same auxin "pumps" (see Leopold, 1969; against this concept, see Burg and Burg, 1966). The auxin transport sites of each side may differ somewhat in number per unit area and/or acitivity, accounting for polarity.

Fluctuations in transport rate were observed (Fig. 8) which might indicate a long term (60 min) oscillation of IAA transport in addition to the short term oscillation with a period length of ca. 25 min, described for Avena and corn coleoptiles by Newman (1963) and Hertel and Flory (1968). In hypocotyl of Helianthus which had been subjected to continuous horizontal exposure, circumnutations have been observed which had a period length about 120 min (Johnsson and Israelsson, 1968). The oscillation curve obtained can be described by the theoretical equation which uses the geotropic reaction times as a constant (Israelsson and Johnsson, 1967). The long term fluctuations of IAA transport rate which are documented here (Fig. 8) might be related to this circumnutation.

Any model of an active, polar auxin transport mechanism should rationalize the following additional characteristics:

(1) The auxin transport system (if the site of exit is limiting) can be locally stimulated in all directions in relation to the gravitational or centrifugal force applied. In the normal gravitational field the basipetal transporting system is not operating at a maximum rate. (2) The system is very sensitive to stimulation i.e. the effect of gravity and of centrifugal force is manifested quickly (10 min). The decay, however, of the local stimulation is slower (see also Hager, 1967). (3) Auxin transport shows 'cooperativity' (Hertel and Flory, 1968), and (4) at least two types of oscillation seem to be possible in the auxin system.

SUMMARY

Basipetal, acropetal and lateral auxin movement is higher in the direction of gravitational and/or centrifugal force than it is in the direction against the force.

Inversion of corn coleoptile sections resulted in a 10-20 percent inhibition of basipetal IAA transport. The inhibitory effect of inversion on basipetal transport of NAA was more pronounced, about 30-50 percent.

Inversion had a quick effect; in 5 mm sections, the inhbitory effect on basipetal NAA transport appeared within 30 min. The inhibition of IAA and NAA transport remained constant throughout a longer transport period. When the tissue sections were returned to the upright position the transport rate increased rapidly, reaching the upright control level after 20-30 min.

The inhibitory effect of inversion may be separated into transport inhibition (exit rate and velocity of the front) and uptake inhibition. The decrease in uptake was much less than the decrease in transport.

When basipetal transport was tested in the direction of gravity and/or centrifugal force, it increased with increasing external force (e.g. 10 x g transport rate was higher than 1 x g). Basipetal transport against the direction of higher centrifugal forces (10 x g) was decreased when compared with control sections (inverted 1 x g).

These results suggest that the geosensors may act by a pressure mechanism.

The rate of acropetal auxin movement seemed to be higher in the direction of gravitational force than against it.

The effect of gravity on lateral auxin transport was shown to be very fast. A significant difference between physically downward and upward lateral movement was seen within 10 min after placing the tissue horizontally.

An oscillation of IAA transport was observed, with a period length of more than 60 min.

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