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Movement of Pulses of Labeled Auxin in Corn Coleoptiles[1]

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Summary. The transit of indole-3-acetic acid through 20-mm sections of corn coleoptiles can be separated from processes involved in the uptake of auxin by the section and the exit of auxin from the section. Aerobic sections are supplied with an exogenous source of ^{14}C IAA for a limited time, and after the source is removed, a pulse of ^{14}C IAA moves down at 12 to 15 mm/hour. After transfer to nitrogen, movement of the pulse at the aerobic rate persists for about 10 minutes; thereafter drops to only 1 to 2 mm/hour and remains at this level during the next 4 hours. Within 2 hours, 70 % of the total ^{14}C in aerobic sections has moved 10 mm or more down the section from the position of the initial peak, whereas after the same time in nitrogen less than 10 % of the total ^{14}C has moved as far.

During the migration down the coleoptile, the peak of radioactivity becomes broader and less distinct. This dispersion is more rapid in aerobic than anaerobic sections, but appears to be nonpolar and to occur along the existing concentration gradients. Diffusion probably contributes to this dispersion.

In both inhibited and uninhibited sections, the movement of the peak, in contrast to its dispersion, is A) polar (downward) and B) independent of existing concentration gradients. Thus transit within the section possesses the fundamental properties of the overall transport system. The reduced amount of transport in inhibited sections is more likely maintained by glycolysis than by a low level of aerobic respiration dependent on the residual oxygen in the tissue.

The transport of indole-3-acetic acid in coleoptiles is of interest because it possesses a high degree of polarity and is independent of the prevailing concentration gradient (11, 12, 15, 16). In the past this movement has usually been studied by supplying the auxin continuously to 1 cut surface of the coleoptile section and recovering the IAA that moves through the section into an agar gel receiving block. In this system, the overall transport necessarily involves: A) uptake from the donor, B) transit through the section, C) exit into the receiver. In this paper, transport refers to movement which can not be attributed to simple physical means; uptake refers to transfer from donor to section and should not be assumed to mean entry into cells.

Uptake (4, 13) and exit (3) are known to be inhibited by anaerobic conditions, but both necessitate passage across injured cells and may involve processes which are not characteristic of transport through the bulk of the tissue. Studies of the movement of applied auxin after it has entered the section are lacking, although this transit should be more comparable to the movement of endogenous auxin than the overall transport from donor to receiver. The effect of reduced oxygen tensions on the movement of endogenous auxin has been studied in shoots of pine (1) and crab apple (9) with conflicting results. To determine if the transit of auxin in corn coleoptiles could be inhibited directly, the transit was separated from both uptake and exit by following the movement of a pulse of labeled auxin down the section. In these experiments, movement of the pulse is sharply reduced by anaerobic conditions but returns to the normal aerobic rate on readmission of air (6). Thus aerobic metabolism is necessary for transit within the section as well as uptake and exit at the ends of the section. This paper presents the evidence that this transit is transport and that in presumably anaerobic sections it can not be totally abolished.

Materials and Methods

Sections 20 mm long were cut starting 3 mm below the tip of etiolated 6 day old corn coleoptiles (8). Each such section was supported vertically with its basal end resting on a receiver block (16 μl) of 1.5 % agar gel and containing 2 % sucrose. During the uptake period, the apical ends of each

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section were covered with similar agar blocks containing 10 μM ^{14}C carboxyl-labeled IAA (New England Nuclear Corporation, 13.5 c/mole) plus 2 % sucrose. Following an uptake period of either 15 or 30 minutes each radioactive donor was replaced by a similar block containing unlabeled 10μM IAA and 2 % sucrose. Fifteen minutes later, the blocks were removed from 4 such sections and the coleoptiles immediately subdivided. At the same time, the sections that were to be subjected to an anaerobic period were also removed from their blocks and transferred to 125 ml side-arm flasks lined with damp paper toweling. In order to remove air from the central hollow and tissues of the coleoptiles the flasks were evacuated to 0.1 atm and released to N_2 (prepurified, Matheson) 5 times in the course of 2 or 3 minutes. Paralleling the sections in N_2, aerobic sections were evacuated 5 times and released to room air. Since all sections were subjected to the evacuation procedure, the inhibition of movement in anaerobic ones can not be attributed to disruption of transport by the sudden pressure changes.

Following the evacuation procedures, the aerobic sections were replaced in room air with their basal surfaces on receiving blocks (containing 2 % sucrose) for a further period of 5 minutes to 4 hours. The sections in N_2 were held for comparable periods during which N_2 passed first through water and then the flask at about 250 cc per minute. Since auxin did not reach the base of these sections, receivers were not supplied. Since a finite time was required in N_2 before movement was inhibited, conclusions about the rate of movement, degree of polarity, and direction of movement with respect to the concentration gradient were drawn only after an initial period in N_2. It is likely that the period in N_2 before onset of inhibition represents the time required to deplete the sections of O_2. Usually one-half hour was allowed for equilibration but periods as short as 10 minutes were sufficient in some experiments (e.g. fig 2).

The sections were manipulated under ordinary room illumination, but during the periods of uptake and auxin movement the chambers containing the sections were shielded from white light by black cloths.

After the period of movement, each section was subdivided by means of a multi-bladed cutter into 10 successive 2-mm pieces. The methods used to determine radioactivity have already been discussed (4). Each block and each 2 mm piece of section were counted separately in Bray's scintillation solvent. Counting efficiency in the liquid scintillation system was approximately 80 %.

The total activity in sections plus receivers was equated to uptake, and the activity in each sample was expressed as a percent of the uptake. The peak of the radioactive pulse was estimated to the nearest millimeter after various times, but since the sections were only subdivided into 2 mm pieces, the average rate of movement of the pulse could not be determined very precisely.

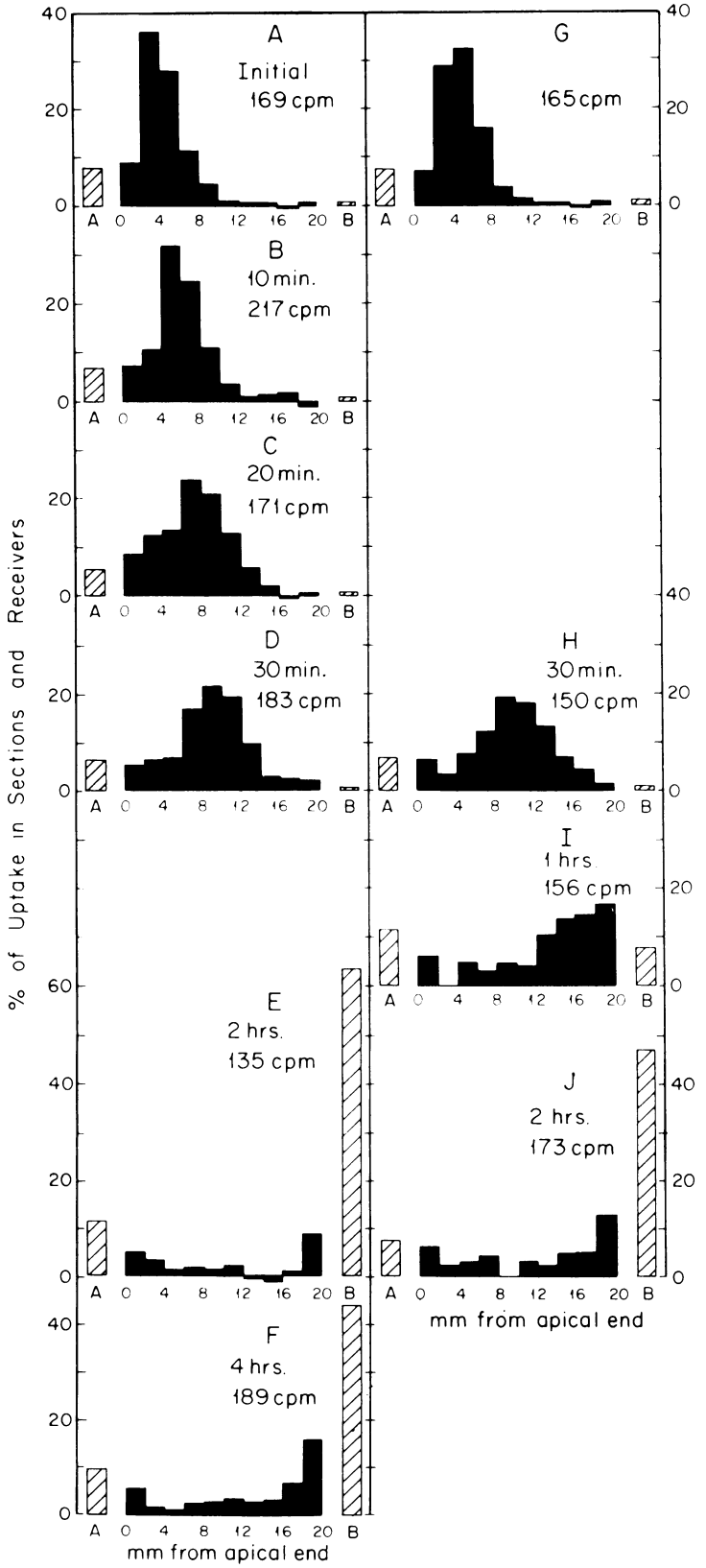
Results

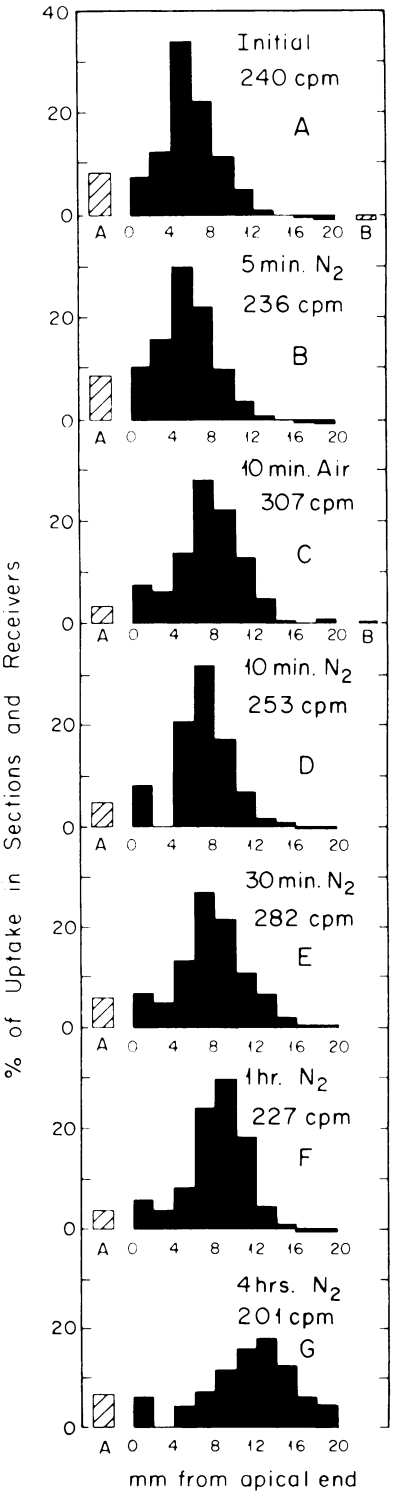
Reproducibility of Experiments. Figure 1 A, D, E, and G, H, J illustrate the agreement between 2 different experiments. In another experiment, 2 sets of duplicate sections were subjected to the same regime, but they were handled separately in different chambers. Not only was the peak activity found in the same piece, but the percent of the uptake in any particular piece of such replicate sets of sections usually varied by considerably less than 10 %. This same level of variation was also encountered between duplicates in the same chamber.

Movement of a Pulse of ^{14}C IAA in Aerobic Coleoptiles. While the donor is present, ^{14}C in the section declines nearly logarithmically with distance from the source (7). This means that at the time the donors are removed, the highest activity in the section is adjacent to the cut surface. Since the radioactivity in the sections does not decline significantly after the ^{14}C donors are removed, (figs 1, 2), destruction of IAA and loss of $^{14}CO_2$ do not contribute to the changes in distribution of ^{14}C IAA. Within 15 minutes after the ^{14}C donor is removed a peak of radioactivity accounting for about 50 to 60 % of the uptake appears well below the cut surface (fig 1A). Its downward movement at 12 mm/hour is readily detected at intervals as short as 10 minutes (fig 1A–D).

By the end of an hour, some 7.7 % of ^{14}C has entered the receiver at the base of the section (fig 1I), and during the next hour the entry into the receiver continues until it contains some 50 to 60 % of the uptake (fig 1E and J); a figure which agrees well with the content of radioactivity in the initial peak. With the exception of the most basal and most apical portion of the section, the ^{14}C is now uniformly low throughout the section; about 1.5 % per mm. The only change in the next 2 hours is that the receiver begins to equilibrate with the basal portion of the section (fig 1F). This effect has been previously noted for sections of *Avena* coleoptiles (4, 5).

Movement of a Pulse of ^{14}C Following Shift to Anaerobic Conditions. In the first experiments at the end of one hour in N_2, the pulse had moved less than 2 mm (6). This movement might represent either the continuation of aerobic movement until the section is fully equilibrated with the N_2 atmosphere or the existence of a sustained low rate of anaerobic movement. To distinguish between these 2 possibilities, the rate of movement of the pulse in sections in N_2 was determined at various


A
Initial
169 cpm
B
10 min.
217 cpm
C
20 min.
171 cpm
D
30 min.
183 cpm
E
2 hrs.
135 cpm
F
4 hrs.
189 cpm
G
165 cpm
H
30 min.
150 cpm
I
1 hrs.
156 cpm
J
2 hrs.
173 cpm
% of Uptake in Sections and Receivers
mm from apical end


Initial
240 cpm
A
5 min. N2
236 cpm
B
10 min. Air
307 cpm
C
10 min. N2
253 cpm
D
30 min. N2
282 cpm
E
1 hr. N2
227 cpm
F
4 hrs. N2
201 cpm
G
% of Uptake in Sections and Receivers
mm from apical end

times (fig 2). The distribution of ^{14}C IAA in the section at the time of transfer to N_2 is shown in figure 2A. During the first 10 minutes of this particular experiment, movement in N_2 does continue at the aerobic rate of 12 mm/hour (fig 2B–D), but then during the next 20 minutes in nitrogen the rate of movement drops sharply so that the profile changes only slightly (fig 2E). During the next few hours, the peak continues to shift slowly (cf. fig 2E, F, G), and this indicates that basipetal movement continues in N_2.

Between 10 minutes and 4 hours after transfer to N_2 (fig 2 and table I), the peak moves downward at 1 to 2 mm/hour. Movement was not detected in 2 of the experiments during a half hour interval (table I) because about an hour is required to detect a significant movement.

The onset of inhibition was rapid. In the experiment in figure 2 it must begin between 10 and 20 minutes, but in others it occurred earlier (6). The time before the onset of inhibition probably reflects the time to deplete the sections of oxygen and the product or products of aerobic respiration that maintain the aerobic rate of transport.

In air, all the moving ^{14}C reaches the receiver in 2 hours (fig 1E, J), but in N_2 none reaches the base of the section in this time. Even after 4 hours the ^{14}C in the receiver is insignificant, and the bulk of the radioactivity is spread from 6 to 16 mm (fig 2G).

Table I. *Average Rate of Movement of Pulse of ^{14}C IAA in Sections in Nitrogen*

Expt	Time after transfer to nitrogen ½–1 hr	1–2 hrs (a) ½–2 hrs (b)	1–4 hrs (a) 2–4 hrs (b)
	mm/hr	mm/hr	mm/hr
11–12	0.0	1.0 (a)	...
2–11	2.0	2.0 (a)	...
3-4	...	1.3 (b)	3.2 (b)
5–6	1.0	...	0.8 (a)
5–2	0.0	...	1.3 (a)
Average	0.8	1.4	1.8

Discussion

Change in Shape of the Peak. The peak not only moves down the section, it also spreads out. Although this dispersion is more rapid under aerobic conditions (cf. fig 1, 2), equilibration of the moving molecules with an immobile phase is apparently not sufficient to account for it. After the bulk of the IAA has moved into the receiver, about 1.5 %/mm of the initial radioactivity remains. In the first half hour, the peak in aerobic sections shifted about 6 mm while the amount of ^{14}C in the 3 pieces of the section with the highest activity dropped from an initial 75 % to about 50 %. In other words the amplitude of the peak decreased at a rate of about 4 %/mm or at nearly 2.8 times the rate of immobilization.

In contrast to the downward migration, the dispersion of the peak appears to be nonpolar and down the existing concentration gradients (fig 1). Since almost all the auxin in the initial peak eventually appears in the receiver (fig 1) diffusion of auxin away from the moving stream or streams followed by its later return to the transport could account for the dispersion of the peak as well as the reduction of radioactivity in the section to its final low level.

Movement of the Pulse is Transport. The term transport has been used loosely in the literature on auxin movement; the appearance of auxin in receivers is often the sole evidence for transport. The auxin detected in receivers may, under certain conditions; e.g., short sections, high donor concentrations, or acropetal movement, have moved through the tissue at least in part by physical means. Operationally the transport can usually be distinguished from other types of movement because it is A) polar, B) at least to some extent independent of the concentration gradient, C) relatively rapid, and D) inhibited by various metabolic inhibitors and specific transport inhibitors (11, 12). The migration of a pulse of ^{14}C IAA down coleoptiles possesses these properties and can rightly be referred to as transport.

The Movement under Anaerobic Conditions. In earlier experiments with oat coleoptile sections,

FIG. 1. (left). Movement of a pulse of ^{14}C IAA down aerobic coleoptiles. Parts A to F present data from 1 experiment; G to J for a separate but similar experiment. The donors containing ^{14}C IAA were removed after 15 minutes and 15 minutes later the sections in part A and G were subdivided. Sections in part B to F and H to J were treated similarly but subdivided after the indicated additional times in air. The histograms give the percent of the section's total uptake in each 2 mm piece and in the basal (B) and apical (A) receivers. The apical receiver was used only during the first 15 minutes after the radioactive source was removed. The average total activity for each group of sections is given. A, G, Average of 4 sections; others, average of 2 sections.

FIG. 2. (right). Movement of a pulse of ^{14}C IAA in aerobic coleoptiles compared to that in coleoptiles transferred to nitrogen. The radioactive donors were removed after 30 minutes and 17.5 minutes later, the sections in part A were subdivided while those in part B, D to G were transferred to nitrogen for the indicated times and those in C were left in air for an additional 10 minutes before subdividing. A-F, average of 2 sections; G, 1 section.

the overall transport from donor to receiver stopped under anaerobic conditions, and the uptake and movement of IAA was nonpolar and apparently by diffusion (4). In the present experiments, movement of a pulse of IAA within the anaerobic sections is sharply inhibited but is not reduced to diffusion. If the only movement were by diffusion, the ^{14}C IAA from the initial peak would be expected to move down the existing concentration gradients (fig 2A) toward both the apical and basal end of the coleoptile. As a result the peak would become less distinct but not shift its position appreciably. Instead although the peak broadens, it continues to move downward at a nearly uniform rate of 1 to 2 mm/hour for at least 4 hours, and almost no activity moves apically from the initial position of the peak (fig 2D–G). The movement of the pulse in anaerobic coleoptiles continues to be polar and independent of the prevailing concentration gradient. Although auxin transport has been inhibited, it has not been completely abolished.

Naqvi et al. (13) report that for corn coleoptiles in either air or N_2, the amount of auxin transported 10 mm in 2 hours is proportional to the uptake and amounts to about 30 to 40 % of uptake. These authors suggest that anaerobic conditions affect the overall transport from donor to receiver by directly inhibiting the uptake. The present work not only demonstrates direct inhibition of transport distinct from uptake (6) but shows that the proportion moving at least 10 mm beyond the initial position of the peak is significantly reduced in N_2 (fig 3). In N_2 about 5 % moved 10 mm within 2 hours, as compared to 70 % in air. The results of Naqvi et al. (13) suggest that their coleoptiles were not sufficiently anaerobic to reduce transport to the extent observed here.

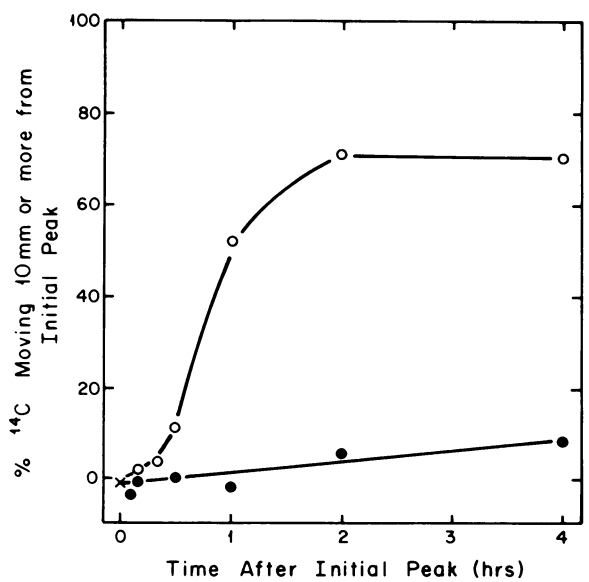


FIG. 3. Comparison of the amount of movement within sections in air and nitrogen. The percent of the total activity in the section that moves at least 10 mm beyond the position of the initial peak is shown as a function of time. Sections in air (○); in nitrogen (●). For purposes of this figure, the 2 mm piece with the highest activity was taken as the initial peak; e.g. piece 2 to 4 mm in figure 1A, but piece 4 to 6 in figures 1G and 2A. Average of data from all experiments.

The movement of the pulse of ^{14}C in the present experiments is more comparable to the movement of endogenous auxin than is the overall transport. While basipetal movement of endogenous auxin was unaffected when sections of pine stems were placed in N_2 (1), it was markedly reduced when stems of crab apple were transferred to atmospheres containing 5 % or less O_2 (9). Either the pine tissues were not completely equilibrated with the anaerobic atmosphere or they have the ability, unlike corn coleoptiles and crab apple shoots, to maintain normal auxin transit under low O_2 tensions.

Since the overall transport in oat coleoptiles is abolished by anaerobic conditions (4), it is apparently more affected than the transit down presumably anaerobic corn coleoptiles. This indicates either that A) transport is completely abolished in oats but not corn or B) that processes at the cut surface are inhibited even more than subsequent transport down the section. In support of the latter possibility is the report (1) that the overall transport from donor to receiver through pine sections is more affected by anaerobic conditions than is the transfer of endogenous auxin into receivers.

Christie and Leopold (2, 3) working with corn coleoptiles report that cellular uptake is not as easily inhibited as the exit of IAA from sections. They suggest that active secretion through the basal membrane of cells is inhibited. It must be pointed out, however, that their experimental procedure would not discriminate between a site of inhibition at the basal membrane and one during passage through the cell protoplast. We may both be dealing with the same phenomenon, and the cellular site of the inhibition is not yet clear.

In our experiments, some transit persists in coleoptiles in N_2. Two possibilities exist to explain this transport: either A) enough O_2 remains in the system to support transport at the reduced rate for as long as 4 hours or else B) glycolysis can supply the energy for the much reduced transport. At present there are no firm grounds for choosing between these 2 alternatives, however, the fact that the rate of movement in N_2 remains about the same during 4 hours, whereas the available O_2 must continue to decrease favors the latter interpretation. Furthermore, growth is reversibly abolished by anaerobic conditions (10, 14), and in the present experiments, the corn coleoptiles during the 4 hour period in N_2 not only failed to grow but even decreased in length by 1 to 2 mm. In terms

of growth, these corn coleoptiles certainly behave as though they are anaerobic.

Although the average rate of downward movement of the peak can not be determined very precisely (6), it is usually 12 to 15 mm/hour in air but only 1 to 2 mm/hour in N_2. The ratio of the minimum anaerobic to maximum aerobic rate (1:15) thus approaches the expected ratio of ATP production (assuming glucose metabolism by the Embden-Meyerhof pathway) of glycolysis to aerobic metabolism.

To summarize, 3 observations lend support to the suggestion that the energy necessary for transport in the inhibited sections is derived from glycolysis. A) The rate of movement does not decline further during 4 hours in N_2, B) growth but not downward movement is totally inhibited, and C) the anticipated reduction in the rate of ATP production is approximately proportional to the maximum reduction in the rate of movement. Since polar movement continues within presumably anaerobic coleoptiles, the previous definition of transport as the component of basipetal movement that requires oxidative metabolism is probably too restrictive. In any case, regardless of the metabolic sequences involved, transport requires expenditure of energy by the living system.

Acknowledgments

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