Acropetal Movement of Auxin: Dependence on Temperature

Abstract. *The amounts of radioactive indole-3-acetic acid that moved, both basipetally and acropetally, through short sections of bean epicotyl were measured at 15° and 25°C in the presence and absence of 2,3,5-triiodobenzoic acid. Low temperature and this second compound each inhibited acropetal efflux of indole-3-acetic acid more than they did the influx. Results are interpreted as supporting a metabolically dependent component of the acropetal movement of auxin in bean epicotyls.*

The movement of auxin in plant stems and leaves is a polar phenomenon in which movement toward the morphological base (basipetal) generally exceeds that toward the apex (acropetal). The degree of polarity is expressed as the ratio of basipetal to acropetal movement. The basipetal component can operate against a concentration gradient and is sensitive to anoxia, temperature, and metabolic poisons; hence it is dependent on metabolic energy (*1*). Goldsmith (*2*) has noted that rigorous proof is still lacking that the basipetal component proceeds against an electrochemical potential gradient and that it is active transport in the narrow sense (*3*).

The dependence of acropetal movement upon metabolism is less well established. Goldsmith (*2*) found that in oat coleoptile sections acropetal movement could be accounted for by passive diffusion; Leopold and his coworkers (*4, 5*) found acropetal movement in various tissues, the polarity varying from weak to strong. Although acropetal movement in sections of bean stems and corn coleoptiles was found to be inhibited by 2,3,5-triiodobenzoic acid (TIBA) (*5*), there is still a need to establish whether such movement represents passive diffusion or whether it is metabolically mediated (*2, 6*), especially in view of the recently proposed model of auxin transport polarity of Leopold and Hall (*7*), which assumes that acropetal movement, either active or passive, does occur.

We found no previous mention of the effect of temperature on the extent of acropetal movement of auxin, and, therefore, we determined transport flux at two temperatures, with and without pretreatment with an inhibitor (TIBA) of basipetal transport. We hoped that the results would indicate whether a purely physical phenomenon (diffusion) was involved or whether the transport was metabolically mediated.

Sections 2 mm long were cut from bean (*Phaseolus vulgaris* L. var. Kentucky Wonder) seedlings (*8*) 3 mm below the primary leaf node by means of a double-bladed cutter. Twenty sections were placed apical end down on each receiver block (*8*) on a microscope slide. A similar block containing 4 μg of ^{14}C-labeled indole-3-acetic acid (IAA-2-^{14}C) per milliliter (*8*) was applied to the morphological bases of the sections and the assembly was placed in a small, dark, moist chamber. The level of auxin used was within the physiologically tolerable range for *Coleus*, according to Jacobs (*9*), and the one that lies below the saturation level of basipetal transport in the bean epicotyl system, as we have shown (*10*). The effect of TIBA on acropetal transport was tested by pretreating the sections with either blank agar disks, or with disks containing 10 μM TIBA, applied to the basal ends of the sections for 2 hours before exchanging them for the radioactive IAA donors. Transport was carried out at either 25° or 15°C for 8 hours after application of the radioactive IAA. Basipetal transport was also measured at 25°C, with and without TIBA. In this case the tissues were placed basal end down on the receiver block, and the donor was applied to the apical end; TIBA was incorporated into the receivers rather than given by pretreatment. Each experiment was carried out three times. At the end of each transport interval, radioactivity in the donors and receivers was determined by scintillation counting in a Packard Tri-Carb scintillation spectrometer. Agar blocks were melted in the counting vials, 15 ml of scintillation fluid (*8*) was added, and the capped vials were stirred vigorously on a Vortex mixer, chilled overnight, and counted.

Table 1. Effect of temperature and TIBA on acropetal uptake and output of IAA-2-^{14}C by stem sections of bean seedlings. Values are given in counts per minute for three separate experiments at each temperature. Averages are in italics, with background subtracted.

Control sections		Sections treated with TIBA (10 μM)	
Uptake	Output	Uptake	Output
Temperature: 25°C			
22,803	807		
25,992	1116	29,474	942
15,442	723	18,690	404
21,405	*882*	*24,082**	*673**
Temperature: 15°C			
16,439	632	19,828	452
18,094	723	22,570	575
12,128	450	16,245	390
15,554	*602*	*19,528**	*472**
Percent change due to temperature			
−27	−36	−19	−28
Q_{10}			
1.45		1.23	

* Percent change from control at 25°C: uptake, +12; output, −24; at 15°C: uptake, +26; output, −22.

Lowered temperature and TIBA each decreased the amount of acropetal auxin output, TIBA having about the same percentage of effectiveness at each temperature (Table 1). Decreasing the temperature also decreased the amount of auxin entering the tissue, but the percentage of reduction in uptake was considerably less than that in output, both with and without TIBA. The addition of TIBA enhanced the rate of uptake, presumably by decreasing the amount normally effluxed to a basal donor by basipetal transport. This effect was twice as great at 15° as at 25°C, attesting to the lowered efficiency of basipetal transport at 15°C. In this system, the ratio of normal acropetal to basipetal output averaged 0.12 and 0.24 for 4 and 8 hours, respectively, at 25°C. At 15°C the 8-hour average was 0.17; 4-hour readings were not taken at 15°.

The Q_{10} values calculated on the basis of total acropetal output were 1.45 for the control and 1.23 for the TIBA-treated sections. These are less than the expected values if acropetal movement is entirely an active metabolically driven process, but are approximately those anticipated if the system is one of passive diffusion. However, the Q_{10} values for basipetal transport were also low and variable, ranging from −1.26 to +1.25 in both treated and untreated sections. These differ markedly from the values of about 3 found for basipetal transport in oat coleoptiles (*11*). The discrepancy may merely indicate a large diffusion component in the bean system. An alternative or additional factor may be that relatively little of the applied auxin is metabolized in the oat coleoptiles (*12*), whereas bean stems conjugate considerable amounts of IAA (*13*). Thus, at lower temperatures less conjugate formation would occur, and the concentration of auxin in the transport stream would be increased. This would tend to offset the decreases induced by

Table 2. Basipetal and acropetal efflux of IAA-2-^{14}C through stem sections of bean seedlings into receivers with and without TIBA at 25°C. Data are counts per minute ± standard error of the mean, with background subtracted.

Expt. No.	Control sections		Sections treated with TIBA (10 μM)		Difference between col. (i) and col. (iv)
	Acropetal (i)	Basipetal (ii)	Acropetal (iii)	Basipetal (iv)	
	Transport time: 8 hours				
5	711 ± 38	4302 ± 51	585 ± 2	570 ± 3	+ 141
6	630 ± 55	2554 ± 42	550 ± 1	508 ± 34	+ 122
7	807 ± 15	2865 ± 41		624 ± 14	+ 183
8	1116 ± 36	5435 ± 70	954 ± 26	942 ± 32	+ 174
9	723 ± 24	2804 ± 49	575 ± 20	645 ± 9	+ 78
	Transport time: 4 hours				
5	497 ± 47	3824 ± 173	366 ± 21	413 ± 7	+ 84
6	284 ± 17	2850 ± 64	271 ± 10	328 ± 10	− 44

lower temperatures on the transport system itself, since the flux must be a function of the concentration of auxin in the transport path at any point along the stem.

Results in Table 2 show that the amount of radioactive IAA entering the acropetal receivers through untreated sections at 25°C exceeded, in six out of seven cases, the amount transported basipetally by similar sections treated with 10 μM TIBA. This concentration caused 81-percent inhibition of basipetal movement at 25°C. If one assumes that passive diffusion of IAA will occur with equal facility regardless of the orientation of the tissue (*14*), then the activity reaching a basal receiver in the presence of TIBA should represent approximately the leakage in the system. Close correspondence of 8-hour acropetal to basipetal efflux from sections treated with TIBA in the receivers indicates that TIBA essentially removed the active transport component. At 4 hours the basipetal movement slightly exceeded the acropetal, suggesting incomplete suppression of active transport. Leakage would thus be slightly overestimated by these data. That the amount of auxin entering the acropetal receivers is about 20 percent greater than the leakage estimated in this way is taken as further evidence for active acropetal transport.

There are numerous problems encountered in attempting to demonstrate unequivocally the existence of an active component of acropetal transport. Poisoning the tissue in various ways can result in decreased acropetal output not only from blocking an active transport process but also from causing protoplasmic changes which hinder passive diffusion.

If the acropetal movement of auxin occurs merely by diffusion and if this movement must operate against the opposing active processes of basipetal transport, binding, and oxidative breakdown, then one would expect that lowering the temperature would increase the amount of auxin that moves acropetally. This would result from the relatively greater sensitivity of the opposing active processes as compared with passive acropetal diffusion. We found no such increase, which further supports the concept that acropetal auxin transport has an active component.

By similar reasoning, blocking active basipetal transport with TIBA should enhance passive acropetal movement. Instead, TIBA inhibits acropetal movement of auxin, which suggests that the latter process is also an active one. This result is not unequivocal, however, because the effect of TIBA might be exerted through modification of the cytological diffusion pathway.

Jacobs (*9*) noted that metabolically dependent acropetal transport can be shown only if the concentration of auxin is not above physiological levels, if the transport interval is not too long, and if surface diffusion is ruled out. By these criteria he found that sections of stems of young *Coleus* transported auxin acropetally one-third as well as they do basipetally (*9*). Acropetal movement of auxin has been reported in petioles, leaves, and roots (*14*), and in fruit pedicels (*15*). Other evidence that acropetal movement of IAA may be metabolically dependent are the findings (i) that TIBA inhibited acropetal as well as basipetal movement of IAA in a variety of tissues (*4*), and (ii) that by removing the receiver blocks from sections of corn coleoptiles at frequent intervals the velocity of acropetal transport (time of first appearance in the receivers) was only slightly less than that of basipetal. The amount moved acropetally increased with age of the tissue (as polarity decreased) and was inhibited by chemical inhibitors of respiration or of basipetal transport (*5*). Although results of our experiments do not prove conclusively the existence of a metabolic component of acropetal transport by the usual criterion of accumulation against a concentration gradient, they, along with the results of others, do strongly suggest that acropetal movement of auxin in bean tissue is not merely a matter of passive diffusion.

Whether this apparent activity reflects only the influence of metabolism-dependent systems on diffusion rates remains to be seen. The concept of a cell's exporting auxin in two directions at slightly different rates (*6*) is more appealing than one that envisions an active transport at only one end of the cell and passive diffusion in the other.

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References and Notes

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8. Seeds were soaked overnight in warm tap water and grown in vermiculite in the greenhouse; they were harvested when primary leaves were fully expanded but the epicotyl above had not begun to elongate (9 to 11 days). Agar blocks were 20 mm in diameter and had a volume of 0.5 ml. The radioactive IAA was purchased from New England Nuclear Corp. and had a specific activity of 2 c/mole. The scintillation fluid consisted of 4 g of 2,5-diphenyloxazole, 50 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, and 30 g of Cab-O-Sil per liter of toluene.
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