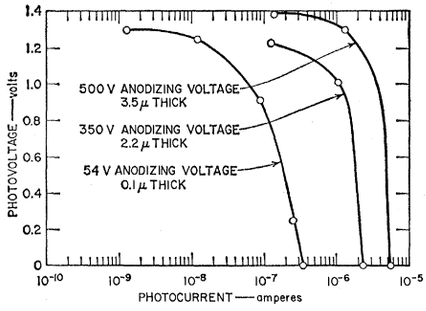

Fig. 1. X-ray induced bulk photovoltaic effect in cells of Ta_2O_5 with Ta and Au electrodes.

posed to ultraviolet irradiation, γ-ray irradiation (from a 1.2-kc Co^{60} source), Sr^{90} irradiation (from a 50-mc source), and 1-Mev electron beam irradiation. All of these irradiations gave an observable bulk photovoltaic effect appropriate to the radiation dose rate.

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Polar Transport of Calcium in The Primary Root of Zea mays

Abstract. *Transport of calcium-45 in 20-mm root segments is basipetal and requires metabolic maintenance. Such transport reaches a maximum rate after immersion of the roots in tracer solution for approximately 12 hours and is still pronounced after 50 hours. Acropetal movement is slight, probably non-metabolic, and essentially constant. Amounts transported are linearly dependent on the absorbing area exposed to tracer solution.*

Ion absorption and translocation in plants has long been known to be a complex sequence of events which can be influenced by transpirational flow. It is generally agreed that the actual absorption of ions is predominantly metabolic while their subsequent upward movement with the transpirational stream is passive. The need for more experiments designed to investigate the catenary nature of this absorption-translocation process has recently been emphasized (*1*). A newly developed method (*2*) of sealing short root segments between two compartments without damaging the roots now makes such an investigation possible. Preliminary results which demonstrate the directional and metabolically mediated transport of calcium in the absence of transpirational flow are summarized in this report.

Zea mays L. var. Peoria was grown in the dark in 0.25 m*M* calcium nitrate solution at 25°C after the manner of Handley, Vidal, and Overstreet (*3*). Characteristically, the roots did not develop root hairs (*4*). Segments 20 mm in length were cut from the primary root of 4-day-old seedlings and sealed into small glass tubes (called root chambers; 1.5-ml volume) as shown in Fig. 1. The seal was effected by compressing a Parafilm-M gasket against the bottom of the root chamber thus forcing some of the sealing compound (5 percent paraffin and 95 percent lanolin) into the bevelled capillary. The segments used were cut at 10 and 30 mm from the apex so that both the apical meristem and the region of elongation were excised (Fig. 1). The segments, therefore, did not elongate during an experiment and presented a cut surface to both solutions regardless of orientation. Because fully differentiated xylem may not always be found in the region 10 to 15 mm from the apex (Fig. 1) (*5*), segments cut from 20 to 40 mm were also tested for orientation-dependent transport of tracer. Both the 10- to 30-mm and the 20- to 40-mm segments showed a pronounced orientation effect (Table 1); therefore, differences in xylem differentiation do not appear to be the basis for the polarity reported here.

Calcium-45 (0.05 μc/ml) was used as the tracer in a solution containing 5 meq/liter of $CaCl_2$ at *p*H 6.3 $\pm$ 0.5. The root segments were immersed in 200 ml of aerated tracer solution until the surface of the solution just contacted the base of the pressure seal. Movement of tracer into the root chamber, which usually contained distilled water, was determined by withdrawing and replacing the chamber solution at 3-hour intervals. The solutions from the root chambers of three similarly oriented root segments were combined, evaporated to dryness, taken up in acidified toluene-ethanol liquid scintillant (*6*), and counted at an efficiency of 54 percent with a liquid scintillation spectrometer.

Movement of calcium in oppositely oriented root segments (10 to 30 mm) showed a pronounced polarity—that is, preferential basipetal transport (Fig. 2). The maximum basipetal transport increment occurred aproximately 12 hours after the segments were immersed in the tracer solution, at which time the basipetal increment was more than 20 times the acropetal increment. After 50 hours the two increments still differed by a factor greater than five. The gradual decline in transport after 12 hours was probably a reflection of a lower metabolic rate resulting from the depletion of respiratory substrate. This polar movement presumably occurred in the absence of transpirational flow, and against both a 5-cm hydrostatic pressure and an osmotic gradient of approximately 0.2 atmospheres. With a 5 meq/liter solution of $CaCl_2$ in the root chamber, the observed incremental transport of Ca^{45} is not statistically different ($p < .01$) from that presented in Fig. 2. The total amount of Ca^{45} retained by the root segments showed no statistically significant differ-

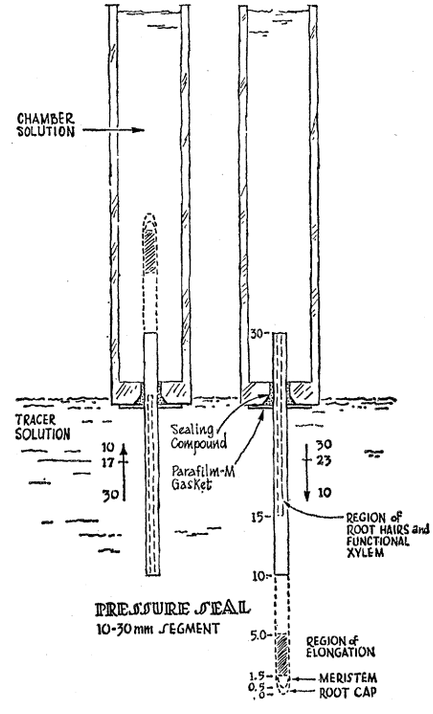

Fig. 1. Cross section of the pressure seal showing the root chamber with sealed 10 to 30-mm root segments in place. Positions of the various regions of *Zea mays* root in relation to the seal are also given. Symbols indicating sealing position (arrows with distance in millimeters to apex) are shown beside the root segments.

ence ($p < .01$) due to segment orientation; thus the polarity of transported Ca^{45} cannot be attributed to an orientation-dependent difference in segment absorption.

After the polar movement of Ca^{45} had been demonstrated, experiments were conducted with segments subjected to various conditions which would inhibit metabolism. 2,4-Dinitrophenol ($5 \times 10^{-5}M$) has been shown to inhibit salt absorption (*7*) without having any significant effects on transpiration (*8*). Dinitrophenol at this concentration (pH 5.8 to 6.4) reduced the total amount of Ca^{45} transported either acropetally or basipetally during a 20-hour period to 10 ± 9 count/min ($n = 8$). With normally transporting segments, the total amount transported in 20 hours is 2239 ± 95 count/min for segments oriented with their apical cuts in the tracer solution (↓) and 142 ± 14 count/min for segments in the opposite orientation (↑). Furthermore, when segments which had been transporting normally for 20 hours were quickly transferred to a tracer solution to which dinitrophenol had been added, the 3-hourly incremental transport decreased by a factor greater than 4 during the first 3 hours after the transfer. Similar segments transferred from 5 meq/liter $CaCl_2$ solution to distilled water at 20 hours showed no significant decline in transport during the first 3 hours. Thus, not only the absorption process but also the transport process appears to be affected. A similar disappearance of transport, and hence segment polarity, was observed when experiments were conducted in an atmosphere of nitrogen (20-hour transport = 48 ± 10 count/min; $n = 8$) or in air at a temperature of 2°C (20-hour transport = 24 ± 11 count/min; $n = 4$). A comparison of these values with those given for normally transporting segments shows that the acropetal movement of Ca^{45} was significantly reduced to segments subjected to conditions which inhibited metabolism. This result could be again interpreted as metabolic mediation of absorption-transport, but it might also reflect permeability changes in the segments (*9*). These experiments demonstrate the metabolic mediation of the absorption-transport process. The desults with dinitrophenol may possibly indicate that the transport process itself is also metabolically mediated.

A pressure drop of one-third of an atmosphere across the seal caused water to flow through the 10 to 30-mm segments at a rate of 0.08 ± 0.03 ml/hr.

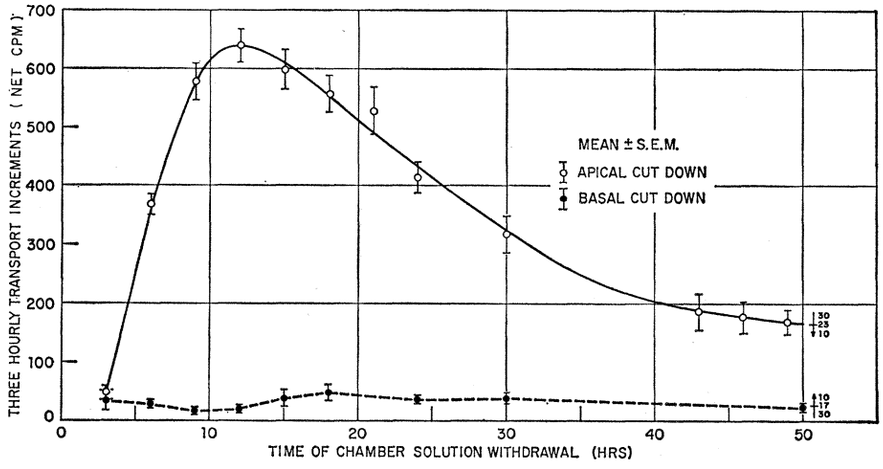

Fig. 2. Incremental transport of tracer calcium (net counts per minute) into distilled water through 20-mm root segments (10 to 30 mm). Symbols to right of 50-hour mark indicate position of segment, see Fig. 1. Upper curve: $N = 21$ except points at 21 hours and 30 through 49 hours for which $N = 8$. Lower curve: $N = 8$.

Under these conditions, Ca^{45} was moved by mass transport either acropetally or basipetally through the segments. If, however, 0 to 20-mm segments were subjected to the same conditions, no water and essentially no Ca^{45} was observed to move during the first 3 hours; thus the integrity of the seal under vacuum was demonstrated. Furthermore, a requirement for cut surfaces at both ends of the root segment appears to be necessary before vacuum perfusion with water is possible under the conditions stated. In another series of experiments 0.1 to 0.2 ml of distilled water was drawn through the segments before they were set up in both orientations for normal transport. No statistically significant difference ($p < .01$) in amounts of Ca^{45} transported in 20 hours (813 ± 156 count/min; $n = 5$) could be observed for segments oriented

Table 1. Transport of Ca^{45} through root segment into distilled water. Net counts per minute (means ± S.E.M.) for groups of three segments are given; where no S.E.M. is given, values are means read from the smooth curves in plots of the data.

Experimental conditions*	No. of roots	Total Ca^{45} transported in 20 hours	3-Hour increments	
			At 5 hours	Maximum increment
10–30 (13) ↑	8	142 ± 14	28	30
10–30 (13) ↓	39	2239 ± 95	257	639
20–40 (13) ↑	4	516 ± 146		
20–40 (13) ↓	5	2571 ± 563		
10–40 (23) ↓	4	5450 ± 617	636 ± 77	1020
10–50 (33) ↓	4	8323 ± 1112	1158 ± 140	1600
10–60 (43) ↓	4	12,315 ± 1173	1703 ± 106	2200

* The first two figures state the distances of the apical and basal cuts from the root apex in millimeters; the parenthetic figure gives the length of the portion immersed in the tracer solution in millimeters. Segment orientation is indicated by arrow showing direction of root apex.

Table 2. Summary of transport data in absolute units. (Reported values are means read from the smooth curves in plots of the data.)

Experimental conditions*	No. of roots	Maximum transport rates (μeq/hr)		Total transport in 24 hours (μeq)	
		Per segment	Per cm² abs. area	Per segment	Per cm² abs. area
10–30 (13) ↑	8	0.00028	0.027	0.0066	0.64
10–30 (13) ↓	21	.0059	.58	.10	10.0
10–40 (23) ↓	4	.0094	.52	.18	9.9
10–50 (33) ↓	4	.015	.57	.26	10.0
10–60 (43) ↓	4	.020	.60	.40	12.0

* See footnote to Table 1.

in either way. Wiersum (*10*) and Smith (*5*) have presented evidence that vacuum perfusion is restricted to vascular tissue; and of the vascular tissue, the end walls of the vessel elements still present in the xylem of 10 to 30-mm segments (*11*) would appear to be the most vulnerable to mechanical rupture by vacuum. This observation, plus the fact that calcium is considered highly immobile in phloem (*12, 13*), would indicate that observed transport takes place primarily in xylem. Apparently, vacuum perfusion with water destroys segment polarity, possibly by destruction of end walls in xylem tissue. Such damage does not seem to occur in 0 to 20-mm segments which possess an intact root apex and hence should not be expected to occur during transpiration in an intact plant.

The effect of segment length was investigated by cutting a series of root segments ranging between 10 and 50 mm in length. The segments were cut at 10 to 30, 10 to 40, 10 to 50, and 10 to 60 mm from the root apex, corresponding to segments of 20, 30, 40, and 50 mm in total length. These segments were sealed with their apical cuts immersed in tracer solution for distances of 13, 23, 33, and 43 mm, respectively. Incremental transport behavior was similar to that shown for 10- to 30-mm segments; the maximum transport increment at 5 hours was consistently equal to or in excess of the maximum increment exhibited by segments only 10 mm shorter (Table 1). Furthermore, not only did the maximum transport increments increase with increasing length of segment immersed (Table 1), but the total amount transported in 20 hours also increased linearly with increasing immersion (Fig. 3). No increase in the total amount of Ca^{45} transported was observed when similar segments of increasing length were sealed so that the length of the portion immersed was constant (Fig. 3). Such behavior would be expected if the transport mechanism were analogous to a conveyor belt supplied by the absorptive processes and therefore implies that the rate limiting process is associated with absorption.

Specific activities of calcium in single root segments and in the root-chamber solution could not be measured accurately because of the very small amounts of total calcium present; but experimental results indicate that specific activity may be considered constant throughout the three-compartment

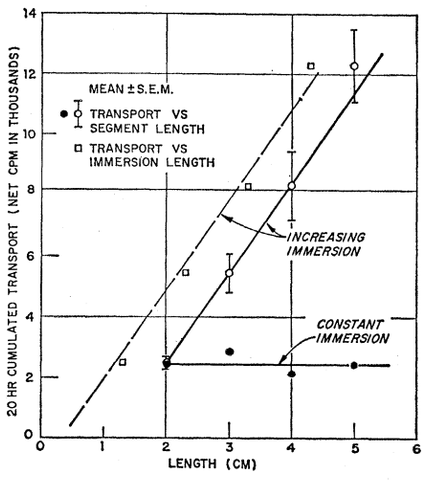

Fig. 3. Total amount of Ca^{45} transported into distilled water during a period of 20 hours. Root segments of various lengths were used; all were sealed with their apical cuts down into the tracer solution. Increasing immersion: $N = 4$.

system defined by the root and the two solutions. First, specific activity was observed to remain constant in the tracer solution, as would be expected since roots do not normally lose calcium under the experimental conditions used (*12, 14*). Also, the amount of Ca^{45} transported into the root chamber was observed to be a linear function of the specific activity of the tracer solution from 5 to 20 μc of Ca^{45} per milliequivalent of total calcium. Second, isotopic dilution during transport within the root does not occur, since the amounts of Ca^{45} transported by segments ranging from 20 to 50 mm in length remain constant when the absorbing area exposed to tracer is constant. Third, the presence or absence of calcium ion in the root chamber has no significant effect on observed transport. Last, the pronounced segment polarity indicates that return from the root chamber solution to the tracer solution is negligible. For these reasons absolute transport rates may be calculated on the basis of the specific activity of Ca^{45} in the tracer solution (10 μc Ca^{45} per milliequivalent of total calcium). The absolute values are presented in Table 2 as total calcium transported per root segment and as total calcium per square centimeter of absorbing area exposed to tracer solution.

The results presented here indicate a metabolically mediated upward absorption-transport process in short root segments which is polar. The transport route is most probably in the vascular tissue. Such metabolic dependence has also been suggested by Biddulph *et al.* (*15*). Kramer (*16*) has indicated that such transport may be particularly important in young seedlings where limited leaf area may preclude high rates of transpiration. The observed transport of Ca^{45} could result from mass transport in fluid movement caused by metabolically generated root pressure. White (*17*) has reported pressures in excess of 6 atm developed by single excised tomato roots, and calcium is often an abundant ion in fluids exuded by root pressure (*18*). The observation that vacuum perfusion destroys segment polarity, coupled with the often reported mobility of calcium only in xylem tissue (*12, 14*), strongly suggests that the observed calcium transport occurs in the xylem. Thus, while the absorption-transport process for calcium in the segments has been demonstrated to be metabolically mediated, further investigation is required to establish definitely whether the linkage is direct or indirect through the mechanism of root pressure.

Unequivocal demonstrations of polarity for ion movement in roots are rare although such movement is implied for noncirculating ions such as calcium (*12, 13*). Organic compounds such as auxins have been shown to move both acropetally and basipetally in roots (*19*); usually, however, basipetal movement predominates, thus indicating an inherent polarity of the tissue. The important matter here is not so much the directional movement of calcium but rather that such polarity can be exhibited by short root segments cut at both ends. The exact mechanisms which produce this phenomenon are not as yet apparent; however, the reported polarity appears incontrovertible. The data also suggest certain additional properties for these root segments. Free space (*20*) cannot be continuous throughout a root segment, since neither metabolic dependence nor the observed polarity are compatible with such a concept. Finally, the linear increase in transported calcium with increasing immersion also suggests a process which includes at least a rate-limiting absorption step followed by relatively rapid upward transport.

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21. I am greatly indebted to Roy Overstreet, Louis Jacobson, and Cornelius A. Tobias for their guidance and constructive criticism during the course of this work. I also wish to thank Raymond Handley and Burton E. Vaughan for their suggestions.

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Toxin from Aspergillus flavus: Production on Food Materials of a Substance Causing Tremors in Mice

Abstract. *A strain of* Aspergillus flavus *produces, on foodstuffs, a substance which causes tremors and convulsions when administered to mice and certain other animals. The tremorgen, which differs from other recognized toxins attributed to this species, is associated with a distinct, dark spot on thin-layer chromatograms viewed under ultraviolet light.*

Strains of *Aspergillus flavus,* when cultured under various conditions, have been reported to produce one or more metabolites toxic for animals. One of these substances, aspergillic acid, was characterized by White and Hill (*1*). While studying methods of purifying the antibiotic flavicin, Bush and his co-workers (*2*) isolated two toxins from culture filtrates, fractions A and B. Fraction A was later identified as β-nitropropionic acid (*3*); fraction B was a yellow crystalline product differing from aspergillic acid in certain physical and toxicological properties. Kojic acid, first isolated from the traditional fermenting rice of the Orient, "koji," is a product of various strains of *Aspergillus,* including *A. flavus* (*4*). We reported that this neurotoxic antibiotic was produced abundantly on corn and other cereal grains inoculated with *A. flavus,* but only traces were detected in animal feeds naturally contaminated by the fungus (*5*). Oxalic acid was also produced by this species on certain feeds (*6*) and often appeared simultaneously with kojic acid in liquid cultures of the fungus (*5*).

English and Dutch workers (*7*) extracted and crystallized two potent hepatotoxins from peanut meal contaminated with *A. flavus.* These were designated "aflatoxins B and G" (proposed B_1 and G_1), according to their respective blue and green fluorescence on paper or thin-layer chromatograms. Asao *et al.* (*8*) presented evidence concerning the molecular structure of these toxins, showing their relationship to synthetic coumarin. A recent report (*9*) described another aflatoxin, designated B_2, which apparently is dihydroaflatoxin B_1.

Milner and Geddes (*10*) reported strains of *A. flavus* to be among the most common fungal contaminants of cereal grains. In our experience this organism has been a frequent isolate from toxic feed, particularly from samples of contaminated feed connected with liver disease syndromes of swine and cattle similar to those described by Burnside *et al.* (*11*).

In a program of screening toxigenic fungus cultures, we found that *A. flavus,* strain QM 6738, when grown on moistened cracked corn, produced an unusual neurological toxin in addition to kojic acid. Both substances were present in crude methanol and chloroform extracts of the experimentally contaminated feed. This new toxin differed from kojic acid and the other recognized toxins of *A. flavus* in certain chemical properties and physiological effects. The characteristic response in mice consisted of tremors, sometimes followed by convulsions, depending upon the dosage. Several other isolates of *A. flavus,* including two which produce aflatoxins, failed to have this effect.

Dublin ICR and SW mice showed signs of illness 10 to 30 minutes after administration by stomach tube of 0.5 to 1.0 mg of the partially purified toxin. This dose represented approximately a 50-fold increase in toxicity over unfractionated methanolic culture extracts. The animals first became inactive but responded to auditory and tactile stimuli and exhibited marked tremors of the entire body when movement was attempted. The trembling became more pronounced in 1 to 2 hours and continued for several hours. Marked improvement was usually evident the day after administration of the toxin, although some mice continued to exhibit tremors and stiffness of the limbs for 2 days. Somewhat smaller doses produced either no perceptible response or only temporary inactivity with mild tremors of short duration.

A dose of 2 mg resulted in characteristic tremors followed, in 1 to 2 hours, by sudden hyperactivity with intermittent convulsions. The slightest stimulus caused affected mice to make rapid paddling movements of the legs without forward progress. Frequently an animal would rise on its hind legs, fall backward, and make several twisting, "log-rolling" motions of its entire body before righting itself. Death sometimes occurred within 2 hours during a tetanic convulsion. Animals dying in convulsions showed marked rigidity of peripheral muscles, observable after death. Mice that survived the repeated convulsive seizures usually continued trembling for 1 to 2 days, but eventually recovered.

The response of mice to intraperitoneal injection was very similar to their response after administration by stomach tube. Guinea pigs and rats were also susceptible to either oral or intraperitoneal administration of the tremorgen.

In mice the response to this toxin is