Translocation of Calcium. Exchange versus Mass Flow [1, 2, 3]

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Introduction

In the field of plant nutrition there are many problems which require, for their solution, an understanding of the distribution of accumulated mineral salts between various plant parts. In their discussion of interrelations of salt accumulation with growth and development of the plant body, Steward and Sutcliffe (14) have provided a background for understanding distribution problems. But, they have concluded that despite the fact that the pattern of distribution is controlled and integrated, the method by which it is accomplished is totally unknown.

In a discussion of upward movement of solutes, Biddulph (2) suggests that adsorptive forces residing in the biocolloids constituting the walls of the conducting channels might operate differentially to regulate the ascent of various ions towards their receiving tissues or organs. This suggestion could be justified from an experiment of Hewett and Gardner (8) showing an adsorption of zinc ions passing through sections of grape canes.

Pursuing the matter further, Biddulph et al. (5) showed that labeled calcium did not ascend the stem of bean plants with the labeled water (THO) of the transpiration stream as would be expected in a mass flow system. Instead, the ascending calcium was shown to participate in exchange and deposition reactions. In short, the data indicated that calcium ascended the stem by an exchange mechanism.

In recent years studies of solute uptake by roots and other tissues have been much emphasized, and the active phase of the process, as it relates to mineral accumulation in tissues, has been quite well described (12). However, agreement concerning the mechanics of uptake by roots of those ions destined for translocation to the shoots has not been attained. One of the questions which remains unanswered is the role of the ascending transpiration stream on both the uptake and release of nutrient ions into the vascular tissues (6, 10, 13, and others).

Since knowledge of the mechanisms of entry into the roots of translocatable ions is limited, the present experiments on the mechanism of ascent were designed in such a way that their interpretation would be independent of the mode of entrance. This was accomplished by studying the ascent of calcium, and the arresting of its ascent, without interfering with the transpiration stream. The experiments were considered to be conclusive when, after arresting the ascent of calcium, it could be started again, at will, by introducing replacement ions of Ca, Sr, and Mg, but not with K. In this respect, the results displayed features which were consistent with exchange reactions, but which were wholly incompatible with mass flow.

Materials and Methods

Bean plants (*Phaseolus vulgaris* L., var. Red Kidney) were grown in half-strength Hoagland's solution (9) with micronutrients and aeration for 12 days after the straightening of the hypocotyl. The growth conditions were: temperature 23 ± 1°, light 1,000 to 1,200 ft-c (fluorescent) on a 12-hour photoperiod, relative humidity 60 ± 5 %.

Plants were then placed, individually, in the above environment into 5 liters of nutrient solution containing about 25 μc of Ca^{45} per liter for varying periods of time. The uptake, or influx, periods were 45 minutes, 1.5 hours, 3 hours, and 6 hours. After treatment some of the plants were replaced in unlabeled nutrient solution, also for varying periods of time. The maximum combined treatment time in each series was 12 hours, all in one photoperiod. Periods in the unlabeled solution were termed efflux periods. For the 45-minute influx period the efflux periods were 3.75, 7.5 and 11.25 hours; for the 1.5-hour influx period the efflux periods were 3.5, 7, and 10.5 hours; for the 3-hour influx period the efflux periods were 3, 6, and 9 hours; and for the 6-hour influx period the efflux periods were 2, 4, and 6 hours.

As the plants were removed from the nutrient solution, the 2.5 cm section immediately above the cotyledonary node was harvested, and the rest of the plant discarded. The 2.5 cm section was divided into wood and bark. The tissues herein referred to as bark are those exterior to the cambium, and those interior to it are referred to as wood (3). The wood and bark sections were analyzed for Ca^{45}. The Ca^{45} was determined by wet digestion of the wood or bark

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section in concentrated nitric acid, and subsequently counting a dried aliquot of the digestion solution.

A series of nutrient deprivation experiments were performed in which the plants were subjected to a period of starvation by placing them in a nutrient solution 1/100 of the concentration of the above (1/200 strength Hoagland's solution), rather than in distilled water, for 24 hours prior to experimentation. The purpose of this action was to clear the tissues of possible pools of accumulated calcium not yet distributed on exchange sites. These plants were then subjected to a 1.5-hour period of influx in 0.005 N $Ca^{45}Cl_2$ solution to saturate the exchange sites. This period was found experimentally to be adequate (fig 1.) An efflux period of 3.5 hours in a dilute nutrient solution followed the influx period to determine whether the tracer would remain in the stem or ascend with the transpiration stream. Some members of this group were then placed in 0.005 N solutions of either $CaCl_2$, $SrCl_2$, $MgCl_2$, or KCl for additional efflux periods of 3.5 and 7 hours to determine which ions would free any arrested tracer for upward movement. Others, the control plants, remained in the dilute nutrient for the remaining period. The 0.005 N concentration was selected because it corresponded to the concentration of the Ca in the half-strength Hoagland's solution in which the plants were grown.

The data represent the average values from 4 plants for each treatment indicated. Standard deviations indicate the variability. A total of 108 plants were required, 68 for the first experiment, and 40 for the second. Plants were handled individually, usually 4 to 8 in number, on the same day each week. Plant cultures were rotated on a weekly basis, so 18 weeks were required for the experiments. With 2 obvious exceptions, i.e., bark data in figure 2, the individual datum points define relatively smooth curves.

Results

The results of the influx-efflux experiments are discussed first.

Influx. Plants were harvested immediately after the termination of the various influx periods in order to obtain time sequence curves for influx into the stem sections. These curves are shown as the dashed lines in figure 1 for the wood and figure 2 for the bark sections. In both of these curves the 2-phase, exchange-accumulation characteristic is evident with the shoulder occurring at about 1.5 hours. This is the type of curve which has been described many times by other workers (7, 12) as representative of intake by adsorption and by metabolic accumulation.

Efflux. Wood. Efflux curves for wood sections are shown as solid lines in figure 1. They appear to be asymptotic in nature. There is a rapid loss of Ca^{45} followed by a decreased loss for a period of about 12 hours at which time the Ca^{45} content remained nearly constant. The course of the efflux curve for the 1.5 hour influx period has been carried out as far as 72 hours without further appreciable change in the Ca^{45} content. During the efflux, the destination of the tracer Ca released from the stem section is upward to the leaves under the influence of the transpiration stream.

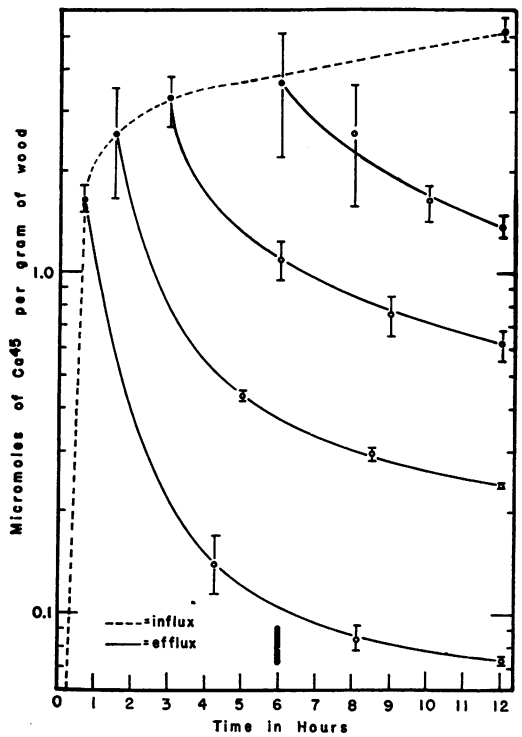
Efflux. Bark. The efflux curves for the bark, shown as solid lines in figure 2, are similar to those of the wood sections except that (a) in the case of the 6-hour influx the efflux curve initially continued to rise for a short period before the characteristic decrease commenced, and (b) in general, efflux was less pronounced in the bark than was the case for the wood. An initial rise in tracer at the onset of other efflux periods might have become apparent had shorter sampling periods been chosen.

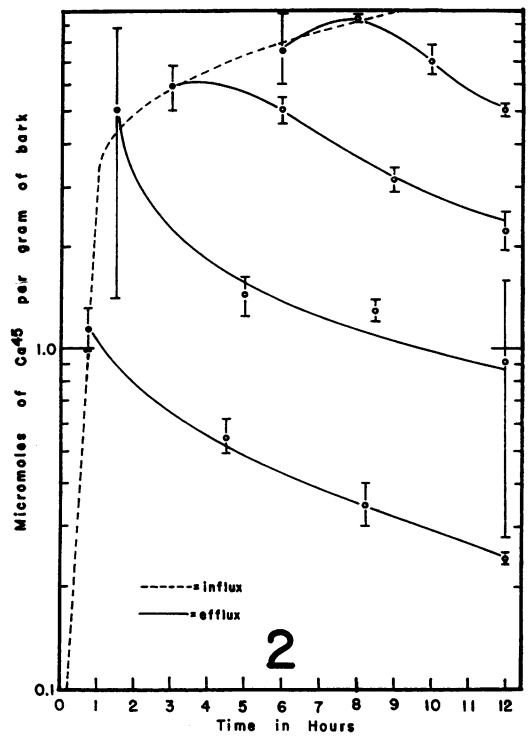
Nutrient Deprivation Experiments. The results of these experiments are shown in figures 3 and 4 for the wood and bark, respectively. The curves for the 1.5-hour influx period are almost identical with those shown in figures 1 and 2. The efflux curves representing tracer loss from the sections during the period when only minute traces of unlabeled ions were available to the roots, differ greatly from those of the foregoing experiments where the plants were maintained in half-strength Hoagland solution. At the beginning of the efflux period, the wood sections increased slightly in Ca^{45} content. Subsequently the wood sections showed only a slow steady loss of tracer against the upward movement of the transpiration stream as long as replacement ions were unavailable via the roots. In the bark sections the Ca^{45} content continued to increase during the initial phases of the efflux period, leveling off at about 8 hours to a high, constant value.

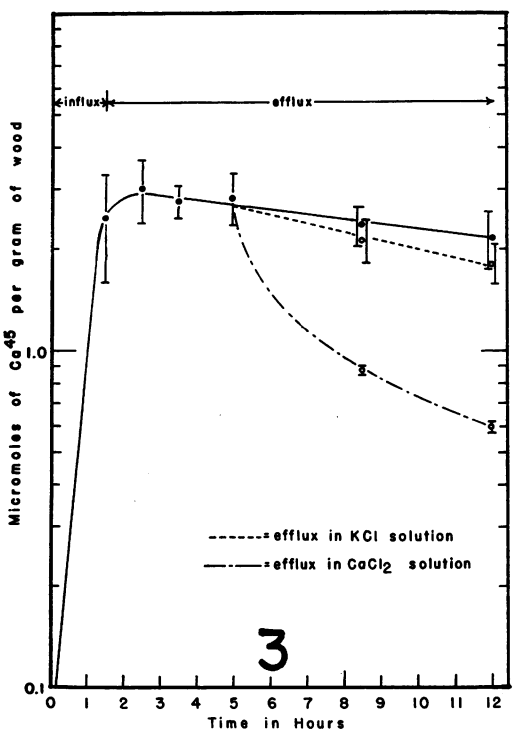
When replacement ions from the 0.005 N salt solutions were made available to the roots, a freeing of the tracer occurred and it exchanged up the stem, providing the replacement ions were in the right position in the lyotropic series to replace the tracer calcium. Unlabeled calcium, strontium and magnesium ions replaced the tracer calcium in this descending order of efficiency. Potassium ions would not do so effectively. Transpirational water alone was also ineffective in moving the tracer calcium up the stem.

Discussion

The experiments were relatively simple in essential design and, apart from certain minor points, are not in need of detailed supplementary analysis. In essence, the uptake, or influx, curves obtained in these experiments were so similar to the well-understood, 2-phased uptake curves for root tissues that the processes represented by each must, of necessity, have much in common. Our influx curves, represented by the dashed lines in figures 1 and 2, showed the 2 phases, whether expressed as the amount of tracer per section, or per gram of tissue. Phase 1 included the course of the reversible exchange reaction until its termination in the saturation of the exchange sites with the tracer. Ascent of







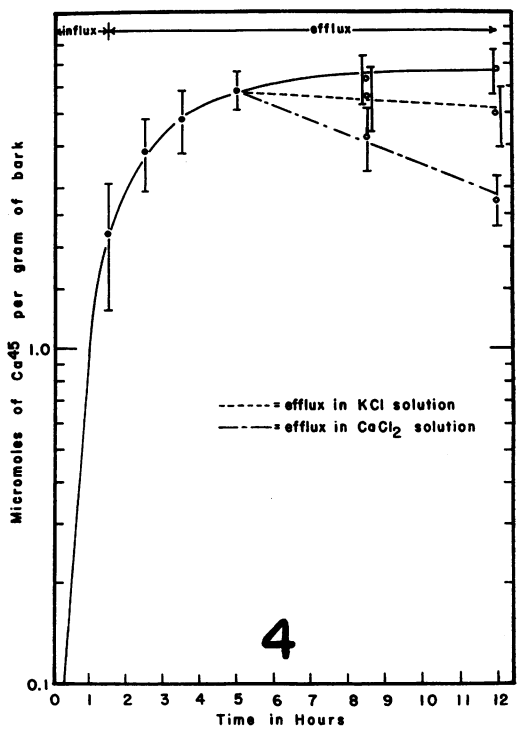


FIG. 1 and 2. Influx of Ca^{45} into a 2.5 cm section of bean stem between the cotyledonary and primary leaf nodes —dashed lines. Efflux of Ca^{45} from the sections—solid lines. Fig. 1, Wood. Fig. 2, Bark.

FIG. 3 and 4. Same as fig. 1 and 2 except: Plants held in dilute nutrient (1/100 strength) for 24 hours prior to treatment; the influx period was 1.5 hours only in 0.005 N $Ca^{45}Cl_2$; the efflux period was 3.5 hours in 1/100 strength nutrient followed by 0.005 N $CaCl_2$, 0.005 N KCl (dashed lines), or 1/100 strength nutrient controls (solid lines). Fig. 3, Wood. Fig. 4, Bark.

the tracer would then proceed by exchange under steady state conditions, the transpiration stream aiding the exchange reaction. To operate in this manner, adsorption on the sites would have to be strong enough to prevent a significant loss of ions to the solvent (transpiration stream), yet weak enough to permit movement along the exchange sites.

The nature of what we have referred to as exchange sites is not known. Jansen et al. (11) however, have indicated that pectic materials present in cell walls of coleoptile tissue could easily account for the total exchange capacity of the tissue. This is not to say that the matter is solved, merely to recall that tissue is known to possess exchange sites.

Phase 2 of the uptake consists of the slow sustained increase in tracer representing the removal of ions from the exchange sites to a more immobile state. This removal has the important consequence of creating vacancies on the exchange sites into which calcium ions might move. Two removal mechanisms include the utilization of ions by actively metabolizing cells of the meristematic regions, and the deposition of ions in the crystal systems of the oxalate type which are found well distributed throughout some of the more mature tissues.

Evidence for a Ca^{45} accumulation in the crystal systems has been presented in preliminary form (1, 4). It is considered proper to relate the deposition of Ca^{45} in the crystals to metabolic accumulation since oxalic acid is a metabolic product and the crystals occur in living cells. The insolubility of the oxalates explains the permanence of the immobilization.

The influx curves for the bark (fig 2) also consist of 2 phases. Phase 1 for the bark is similar to phase 1 for the wood except that the former displays a time delay for the beginning of uptake. For instance, at the 45-minute period the bark is in the initial stages of phase 1 uptake whereas the wood is nearer the completion of this phase. This would indicate that the Ca^{45} ascended in the wood and was laterally transferred to the bark. Lateral transfer has been shown to be more pronounced at the leaf nodes with the probability that some of the transferred ions then descend in the phloem (5). Regardless of the manner of entry into the bark, it is clear that the process displays a reversible exchange phase and an accumulation phase.

The efflux curves represent the loss of tracer from the sections, the destination of the tracer being upward to the leaves in the normal direction of transpirational water movement. We have, in effect, used this normal movement of transpirational water to aid the upward exchange of the nontracer calcium ions, which then replace the tracer calcium. The family of efflux curves obtained is characteristic of those which in root studies have been regarded as representing the loss of ions by the reversible exchange process. The curves appear to be asymptotic to a family of lines parallel to the x-axis, each of which would intercept the y-axis at a point representative of the value for the unexchangeable Ca^{45} fraction. The curves do not become parallel with the x-axis in 1 photoperiod (transpiration period); however, some of them approach this condition sufficiently close to disclose the nature of the curves. Since the efflux from the sections was, in part, dependent on the transpiration stream moving the ions, the efflux periods were not extended into the dark period when the ascent of the transpiration stream would be curtailed.

The efflux curves for the bark show not only that the exchangeable fraction of the tracer was smaller, but that it moved from the section more slowly than was the case for the wood. Apparently movement back from bark to wood was accomplished with greater hazard and less speed than the original transfer from wood to bark. This also indicated that deposition was a more pronounced characteristic of bark than of wood, and that exchange capability was a more pronounced characteristic of wood than of bark.

It should be noted that after the longer influx periods, the efflux from the bark did not begin sharply; rather, influx was carried over into the efflux period. This is thought to be due to the continued lateral transfer of adsorbed Ca^{45} to the bark until the lower boundary of the mobile tracer aliquot had ascended beyond the stem section under examination.

In the nutrient deprivation experiments it was possible to introduce the tracer into the stem, hold it there by depriving the roots of a source of ions for ascent—while the transpiration stream continued to flow—then release the tracer ions for ascent supplying those ions in the proper position in the lyotropic series to replace them. Calcium, strontium, and magnesium ions freed the Ca^{45} for ascension at a rate consistent with their relative positions in the lyotropic series. Potassium ions were almost without effect as would be expected from the position of potassium in the series.

Finally, on the basis of the first experiment in this study, it would appear justifiable to conclude that calcium ions ascended the stem by an exchange reaction on the biocolloids of the conducting tissue. This would also be expected on purely theoretical grounds. Further, under the conditions of the second experiment, not only is the exchange mechanism called for as an explanation of the ascent of calcium, but the possibility that ascent was by mass flow has been excluded.

This leads us to a concept of calcium ascent that is based not on the loss of water from the individual parts, but on the metabolic removal of the ions from the exchange columns leading to the individual parts. This permits the various tissues to acquire nutrient ions in proportion to their metabolic utilization rather than in proportion to their transpirational rates, as is the case with mass flow. Just how general this method of ascent might be is unknown, but preliminary work with K^{42} and S^{35} showed that much remains to be done before a generalized picture of the mechanics of ascent can be obtained.

Summary

Bean plants (*Phaseolus vulgaris* L. var. Red Kidney) were grown in nutrient solution, then placed in Ca^{45}-labeled nutrient solution for varying periods to obtain intake, or influx, curves for a selected stem section. Plants treated similarly were returned to non-labeled nutrient solutions for varying periods to obtain a series of Ca^{45} efflux curves.

Influx curves consisted of 2 phases, a reversible-exchange phase and a metabolic-accumulation phase similar to the curves obtained in root studies.

Efflux curves, representing the replacement of the exchangeable Ca^{45} by nonlabeled calcium, were asymptotic in nature, as is typical of exchange reactions.

A second experiment was performed with plants held in 1/200 Hoagland solution for 24 hours, given $Ca^{45}Cl_2$ for 1.5 hours, then placed again in 1/200 Hoagland solution for 3.5 hours. The flow of transpirational water did not cause the ascent of the Ca^{45} during the latter period, but the tracer was quickly freed for ascent by making available to the roots $CaCl_2$, $SrCl_2$, or $MgCl_2$. KCl would not replace the Ca^{45}, freeing it for ascent.

The data indicated that the tracer calcium moved up the stem by a process of exchange. This concept of upward translocation in the plant stem is in controversy with the classical concept of mass flow.

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