

ABSORPTION AND MOVEMENT OF RADIOPHOSPHORUS IN BEAN SEEDLINGS

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Experimentation

Preliminary results using the radioactive isotope of phosphorus (P^{32}) as a tracer atom in Red Mexican beans (2) demonstrate the value of the method and its general application to plant physiological problems. Whenever it is desirable to follow the path or metabolism of single atoms, this procedure will prove extremely valuable.

The equipment used for the detection and measurement of the radiophosphorus consisted of a NEHER-HARPER high speed Geiger counter circuit (14) in connection with a direct-reading counting rate meter for random pulses as designed by GINGRICH, EVANS, and EDGERTON (5). A Geiger counter tube with a very thin glass window (4) proved satisfactory. The samples to be measured were held in cellophane cones which in turn were suspended in the concave sensitive area of the counter tube. Only a relatively small percentage of the total beta radiation emitted by the radiophosphorus entered the cathode cylinder of the counter tube; however, the geometric conditions remained constant for all determinations, and the counts per minute recorded represent a constant percentage of the total emanation. Results represent comparative amounts rather than absolute quantities.

The radiophosphorus was obtained through the courtesy of Dr. E. O. and Dr. E. H. LAWRENCE, of the Division of Radiation, University of California. It was made by bombarding 8-million volt deuterons against red phosphorus, which in turn was converted into sodium phosphate. The radiophosphorus-containing nutrient solution was made as follows:

Na ₂ HPO ₄	0.0075 M (contains the radiophosphorus) ¹
KCl	0.0020 M
Ca(NO ₃) ₂	0.0020 M
MgSO ₄	0.0010 M

The solution received continuous aeration. The conditions were not favorable for very rapid transpiration, as the light intensity fell from 2000 to 200 foot candles during the experiment. The temperature varied between 21° and 24° C., but was for the greater part of the experiment between 22° and 23° C.

Bean seeds, variety Red Mexican, were germinated and the plants grown in a complete nutrient solution until the fifth alternate leaf was unfolding.

¹ The final nutrient solution contained 0.76 $\mu\text{c}/\text{cc}$. of radiophosphorus.

They were then sorted for uniformity and three selected for tracer work. The first plant was placed in the radiophosphorus-containing nutrient solution for one hour, the second for two hours, and the third for four hours. After the required time, each was removed, the roots washed carefully and the plant dissected, dried in a 75° C. oven, ground to a fine powder and 10 mg. samples weighed for analysis. The remainder of the root tissues of each plant was extracted with ether in a Soxhlet, and then dried and extracted for three hours with successive portions of hot distilled water. The ether extract was evaporated, and determinations of radiophosphorus made on the residue. Aliquots of the water extract were also assayed. The results are shown in table I.

TABLE I

DISTRIBUTION OF RADIOPHOSPHORUS IN BEAN PLANTS GROWN IN NUTRIENT SOLUTION CONTAINING 0.76 $\mu\text{C}/\text{CC}$. RADIOPHOSPHORUS
(EXPRESSED IN COUNTS / MINUTE / MG. OF FRESH TISSUE)

PLANT ORGANS	TIME IN NUTRIENT SOLUTION												
	1 HOUR			2 HOURS			4 HOURS			COMBINED ROOTS			
	TISSUE	ETHER	WATER	TISSUE	ETHER	WATER	TISSUE	ETHER	WATER	WATER	ETHER	2% HCL	INSOL.
Roots	8.3	0	2.7	25.3	0.07	5.7	33.5	0.15	10.5	29%	1%	61%	9%
Hypocotyl and stem	0.0			1.1			0.6						
Opposite leaves	0.0			0.0			0.9						
1st alternate leaflets	0.0			0.0			0.5						
2nd " "	0.0			0.0			0.8						
3d " "	0.0			0.0			0.8						
4th " "	0.0			0.0			1.2						
5th " "	0.0			0.0			1.3						
Nutrient solution			11.8			11.0			10.4				

After finding that the water extraction removed only a comparatively small percentage of the total radiophosphorus, it was considered desirable to investigate the remainder. The tissue residues from the three roots were combined and extracted with 2 per cent. HCl. The phytin fraction is normally removed by this procedure and determined quantitatively by titration (1). The presence of ferric iron in the tissues, however, caused the precipitation of the insoluble iron-phytic acid complex, which remained in the tissue residue and consequently was not determined as such. The distribution of radiophosphorus in the combined roots can be calculated, and is as follows:

Water soluble	29%	2% HCl	61% (not phytin)
Ether soluble	1%	Insoluble	9%

The water soluble radiophosphorus in the tissues approaches that in the nutrient solution at the end of a 4-hour period.

Discussion

These results are of interest because the method employed enables the experimenter to follow absorption, movement, and accumulation of a nutrient salt in an intact plant.

Table I shows that the uptake of phosphorus under the conditions of the experiment was quite rapid. Radiophosphorus could be detected in the top-most leaves of the plant, a distance of approximately one meter from the base of the hypocotyl, within four hours after the roots were placed in the radiophosphorus-containing nutrient solution. This rate of uptake and movement is not as rapid as that reported by CRAFTS and BROYER (3) who detected Br in the xylem exudate of squash within 30 minutes after exposure of the roots to a solution containing 800 p.p.m. of KBr. With the bean plant, radiophosphorus was detected in the basal part of the stem within two hours after administration of radiophosphorus. It is very interesting to note the large amount of total radiophosphorus which was associated with the roots of the bean plants before appreciable quantities moved upward in the stem. It is not advisable to consider the total amount as being absorbed phosphorus, as relatively much may have been merely adsorbed on the surfaces. That which may be removed by hot water may represent more nearly the fraction which is normally free to move into the xylem vessels, and it can be seen from table I that the water soluble radiophosphorus associated with the roots approaches that in the nutrient solution after four hours of absorption. The relationship of the various fractions studied to the concentration of radiophosphorus in the nutrient solution can be seen from table I. It can also be seen that the time required for the movement of radiophosphorus into the stem corresponded fairly well with the time at which the total radiophosphorus associated with the roots reaches the same concentration as that in the nutrient solution. The proper interpretation of this point is questionable because of limited data.

The path of movement of radiophosphorus in the stem is unquestionably the xylem (15). It is impossible to account for the distribution throughout the plant assuming any other path. If movement were through the phloem (6) the concentration gradient in the aerial parts would necessarily be the opposite to that actually found. It appears, from the small amount of radiophosphorus in the hypocotyl (2) that on arrival of the radiophosphorus in the xylem tissue it is "swept" into the aerial parts by the transpiration stream. The distribution of radiophosphorus in the aerial parts corresponds very favorably to the transpiration rates of various leaves (13).

HEVESY, LINDERSTRØM-LANG and OLSEN (7, 8) present data from which

they contend that phosphorus migrates from leaf to leaf within the plant. They grew sunflower plants in a complete nutrient solution until a first set of leaves was formed, then transferred them to a nutrient solution containing radiophosphorus while a second set of leaves was formed. They then reason as follows:

“ . . . then we must distinguish between two extreme cases: (a) the phosphorus atoms do not migrate; (b) the phosphorus atoms migrate. In case (a) labeled phosphorus atoms should only be found in the upper leaves; in case (b) the labeled phosphorus atoms should be equally distributed between the upper and lower leaves . . . ” (8).

Analysis showed that radiophosphorus moved into the lower leaves as well as the upper ones. This is, of course, no evidence for exchange of phosphorus atoms from leaf to leaf as the authors contend. It is merely evidence that phosphorus moved directly into the lower leaves as well as the upper ones through the vascular tissues supplying those leaves, and not that the radiophosphorus moved into the upper leaves only, and was then exchanged to the lower leaves. The same criticism applies to their work on maize (8). The above experiments were not properly designed to show migration of phosphorus from leaf to leaf. It is possible, however, for phosphorus to migrate from one leaf to another (11) chiefly upward, but it should be emphasized that the data of HEVESY *et al.* are not concerned with this type of exchange. A most interesting relationship between the work of MACGILLIVRAY (11), on the re-utilization of phosphorus by the tomato plant as the supply to the roots is removed, and the work of MASON and MASKELL (12) on the movement of phosphorus in the cotton plant, can be shown. The latter authors demonstrate that after the delivery of phosphorus to the leaves, via the xylem, there may be a downward movement through the phloem. They state that the downward movement in the phloem may be in excess of the amount required by the roots, and some of the mobile phosphorus may find its way back into the xylem and reascend the stem. This is the type of evidence which should be used to demonstrate migration of phosphorus atoms from leaf to leaf, and it is the only sense in which the term “migration” has meaning. No attempt seems to have been made to correct the erroneous impression of HEVESY *et al.*, as the inference is repeated in the new edition of “A Manual of Radioactivity” (9). It is hoped that, by calling attention to the above interpretation, a more correct appraisal of radiobiological activity may be made.

In tracing the movement of radiophosphorus in the bean plant, a concentration gradient has been found which corresponds to a transpiration differential. Accordingly, the transpiration stream delivers phosphorus to the leaves, where it accumulates as water is evaporated. This steepens the gradient in the phloem and allows a downward movement through that

tissue. If the supply of phosphorus to the root is suddenly removed, a diffusion of mobile phosphorus toward the xylem vessels would take place, and upon entry into the transpiration stream the ions would be "swept" upward again. This system of forces and gradients would account for the movement of ions which are water soluble, and which are known to accumulate in the upper parts of the plant as the supply to the roots is reduced or removed.

The very small amount of radiophosphorus in the ether soluble fraction within four hours after exposure to the nutrient solution is interesting, but previous work by WEBSTER and DALBOM (16) has shown that lipid phosphorus constitutes only a small fraction of the total phosphorus of the mung bean. HEVESY and PANETH (9) state that radiophosphorus can enter into the phosphatide molecule only during its synthesis (in animals), and no evidence for the exchange of a newly acquired phosphorus atom for one already in chemical combination in a phosphatide molecule could be found. In the experimental work with the bean plant, it is highly improbable that ample time for much phospholipin synthesis was allowed, as the concentration of ether soluble radiophosphorus is still increasing at the end of four hours, the maximum period of contact with the nutrient solution.

Summary

1. The movement of radiophosphorus was traced throughout the bean plant. The total phosphorus associated with the roots was twice as great as the concentration in the nutrient solution before appreciable quantities entered the aerial parts.
2. The amount of water soluble phosphorus in the roots at the end of a four-hour period was equal to that in the nutrient solution.
3. Movement was rapid, and followed the transpiration stream.
4. Accumulation was greatest in the uppermost leaves.
5. An explanation of movement and accumulation is offered.
6. Criticism of the interpretations of HEVESY, LINDERSTRØM-LANG and OLSEN is offered.

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