THE ABSORPTION AND LEAKAGE OF PHOSPHATE FROM AN EXCISED RICE ROOT AS AFFECTED BY THE OSMOTIC PRESSURE OF THE EXTERNAL SOLUTION[1]

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The absorption of phosphate by plant roots, excised tissues and lower organisms has been reported by several workers (Bieleski, 1966, Clarkson, 1966, Goodman and Rothstein, 1957, Hagen *et al.*, 1957, Jackson *et al.*, 1962, Hopkins, 1956, Leggett, 1961, Leggett *et al.*, 1964, 1965, Lundegårdh, 1958). It is generally agreed that phosphate is accumulated by a metabolically dependent uptake.

All plant parts and especially thin sections derived from them have been observed to leach solute upon immersion in water (Helder, 1956). A large amount of exosmosis of cell constituents was found to be induced by inorganic salts and organic substances (Stiles and Jorgensen 1915, 1917). Helder (1956) mentioned that phosphate and other inorganic salts were usually present in the exudate of roots and other plant tissues along with easily diffusible parts of the cell constituents as the consequence of increased permeability of cell membrane induced by salts, toxic materials or metabolic inhibitors. Burg *et al.* (1960, 1964) reported that exposure to water caused apple, banana, potato and pea sections to lose solute. A high concentration of glycerol or other osmotic agents prevented exosmosis from these tissues. Shen and Shieh (1966) reported that when plasmolyzed rice roots were immersed in water, a great portion of the absorbed phosphate leaked out of the roots. This report presents further information on the absorption and exosmosis of phosphate of excised rice roots as affected by the tonicity of the external solutions.

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Materials and Methods

Preparation of root materials: Seeds of a paddy rice variety, Taichung No. 65 were soaked in tap water at 28–30°C for two days. The seminal roots were cultured and harvested for experimental uses as decribed previously (Shen and Shieh, 1966).

Absorption experiments: Two mM KH_2PO_4 solution was prepared for the absorption experiments. One gram of fresh root was immersed in 100 ml of the KH_2PO_4 soulution for a period of 3 hours. Radioactive phosphate was added to the solution at the beginning of the absorption period. The solution had a pH of about 4.2–4.5. The solution was vigorously aerated and was kept at 30°C. After the 3-hour absorption period, root samples were separated from solution with a copper screen, rinsed with 300 ml of water, spread evenly in planchets and dried at 40°C.

Exosmosis experiments: Two grams of excised rice roots were allowed to absorb phosphate for 3 hours in 2 mM KH_2PO_4 solution which was labelled with radioactive phosphate. The roots were then treated with solutions of different osmotic pressures. Exosmosis of phosphate from the root was expressed either by the radioactive phosphate remaining in the root after the treatment or by the amount of radioactive phosphate leaked out of the root into the external solutions. In the latter case, 0.1 ml aliquot of the external solution was drawn at different times during the treatment for radioactivity counting.

Radioactive assay: The samples were counted with a thin window (1.4 mg/cm^2) Geiger-Müller tube connected to a conventional scaler. In all experiments, duplicated samples were taken and measured.

Paper chromatography: Qualitative studies on the phosphate compounds in rice root and those leaked from the root were carried out with a paper chromatographic technique. The 80% ethanol extracts of rice root or its leakage was spotted on Whatman No. 1 filter paper. The chromatograms were developed one-dimensionally using butanol-propionic acid-water (100:50:70 v/v).

Results and Discussion

The uptake of phosphate by both turgid and plasmolyzing rice roots is shown in fig. 1. Rice root absorbed phosphate ion in a similar manner as barley (Leggett *et al.*, 1965) to absorb phosphate linearly with time for a period of at least 5 hours. During the first 30 minutes, rice root absorbed more phosphate in the solutions containing 0.4M of mannitol regardless of the presence or absence of DNP. This increased rate of phosphate absorption by the plasmolyzed root may be attributed to the increased volume of free space. Plasmolyzing rice root accumulated phosphate ions metabolically, though at a

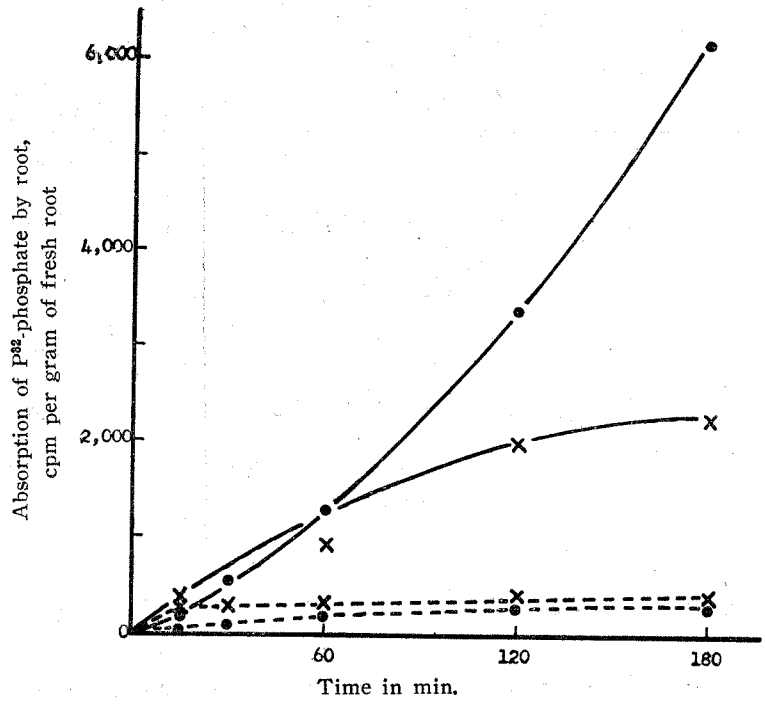

Fig. 1. The absorption of phosphate by excised rice roots in 2 mM KH_2PO_4 solution with or without the presence of DNP and mannitol. ●, without mannitol; ×, with 0.4 M mannitol; —, without DNP; ···, with 10^{-4}M DNP. Roots which were used for the DNP treatments were pretreated with 1×10^{-4}M DNP for 30 minutes before the absorption period.

lower rate than that of the turgid root. Active accumulation was effectively inhibited by the presence of 1×10^{-4}M DNP in the absorption solution.

When rice roots were treated with consecutive soakings in water and 0.4M mannitol solution, a great portion of the absorbed phosphate was lost from the roots if the roots were plasmolyzed first in 0.4M mannitol solution and were then transferred to water (Table I). Measurements of the radioactivity in the external solutions showed that a small amount of absorbed phosphate was present in the mannitol solution while a great amount of phosphate was present in the water when the plasmolyzed roots were transferred to it. Further work showed that rice root plasmolyzed in the hypertonic solution of inorganic salts ($NaCl+Na_2SO_4$) resulted in a great loss of its phosphate when it was transferred to water. Polyethylene glycol (C20M) solution of the concentration of 22.8% by weight which would have an osmotic pressure of about 10 atm. as determined with thermocouple psychrometer method by Lagerwerff *et al.* (1961) induced neither appreciable plasmolysis nor great phosphate leakage of rice root.

Table I. *The loss of absorbed phosphate from rice roots as caused by consecutive soakings in water and 0.4M mannitol solution*

Treatment*	P^{32}-phosphate remaining in root, % of control
control ←1 hr→	100.0
A	86.3
B	97.5
C	25.6
D	27.6
E	88.4
F	91.6

* absorption
mannintol 0.4M
water

It was noticed that a loss of dry matter from the root always accompanied the leakage of phosphate (Table II).

Table II. *The loss of dry matter and absorbed phosphate into water from rice roots plasmolyzed previously in different hypertonic solutions*

Treatment	Dry wt. of root after treatment, % of control	P^{32}-phosphate remaining in root, % of control
control	100.0	100.0
salts 0.4M	90.2	54.2
mannitol 0.4M	80.9	33.4
C20M 22.8%	102.7	87.1

The data presented in Table III show that rice roots plasmolyzed in 0.5M mannitol solution lost more phosphate when they were subsequently soaked in water (M5–0) than the rice roots plasmolyzed in 0.3M mannitol solution (M3–0). Plasmolyzed rice roots lost more phosphate into the water (M5–0, M3–0) than into 0.2M mannitol solution (M5–2, M3–2). These data suggest that the leakage of phosphate is proportionally related to the degree of plasmolysis of the root and the rapidity of water entry during deplasmolysis. The experimental results summarized in Table IV indicated that when the concentration of the external solution was first gradually diluted from the 0.4M solution to 0.2M, the plasmolyzed root lost less phosphate than when it was directly transferred from the 0.2M solution to water.

Table III. *The loss of absorbed phosphate from rice root as caused by consecutive soaking in solutions of different tonicities*

Treatments*	P^{32}-phosphate remaining in root, % of control
Control	100.0
M5-0	20.6
M5-2	34.3
M3-0	63.6
M3-2	82.2
W	94.4

* absorption; mannitol 0.5M; mannitol 0.3M; mannitol 0.2M; water

Table IV. *The loss of absorbed phosphate from rice root as affected by the rate of deplasmolysis**

Period for changing down from 0.4 to 0.2M mannitol solution (hr.)	Interval between successive additions of water (min.)	Decrease in the concentration of mannitol solution caused by each addition of water (M)	P^{32}-phosphate remaining in the root (% of control)
1st experiment			
8	60	0.025	46.9
4	30	0.025	39.2
2	15	0.025	35.6
0**	—	—	23.9
2nd experiment			
4	15	0.0125	55.1
4	30	0.025	53.8
4	60	0.05	48.7
4	120	0.1	43.3
0**	—	—	28.4

* Rice roots were prefed with phosphate and then soaked in 0.4M mannitol solution for 1 hour. Afterward, the concentration of the mannitol solution was diluted at different rate from 0.4 to 0.2M by means of adding water as indicated in the table. The roots were transferred from 0.2M mannitol solution to water and were soaked therein for 1 hour.

** Roots directly transferred from 0.4M mannitol solution to water.

An example of the time course of phosphate leakage from plasmolyzed root into water is shown in fig. 2. In all of the cases studied, the initial rate

of phosphate leakage and the time needed for reaching equilibrium were affected by the difference between the osmotic pressures of the solutions of consecutive soakings and the amount of absorbed phosphate in the root.

The phenomenon of the leakage was further studied by measuring the rate of phosphate exosmosis at different temperatures in the range from the freezing point to 40°C and in the solution of different non-radioactive phosphate concentrations (fig. 3). In the temperature range of 20–40°C, the rate of phosphate

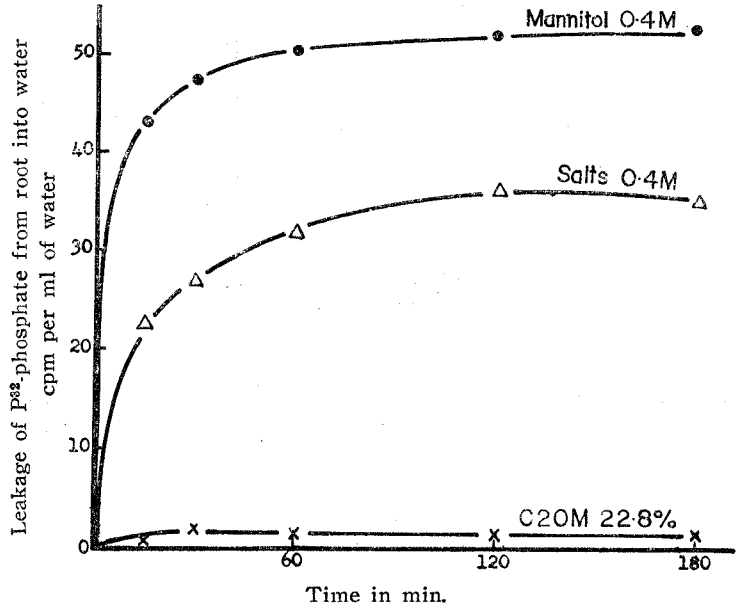

Fig. 2. The leakage of absorbed phosphate into external water from rice roots plasmolyzed previously in different hypertonic solutions.

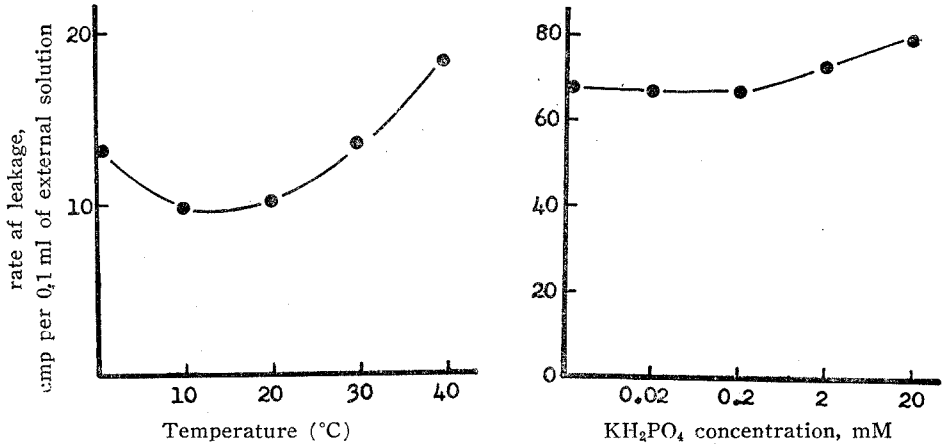

Fig. 3. The rate of leakage of absorbed phosphate from plasmolyzed rice root as affected by temperature (left) and the concentration of phosphate in the external solution (right). The rate of leakage was expressed by the amount of phosphate leaked from the roots into the external water or KH_2PO_4 solution during the first 10 minutes of soaking.

leakage was a linear function of temperature with a temperature coefficient of about 1.3, typical of a diffusion process. However, the rate curve flattened between 10 and 20°C, and rose when the temperature was approaching the freezing point. The rate of phosphate leakage was not affected by the presence of the same ion species at low concentrations in the external solution. However, it was somewhat increased when the concentration of phosphate in the external solution reached 2–20 mM. It is speculated that the phosphate leakage is essentially a diffusion process. Its rate was enhanced near the freezing point by the changes in certain physical properties or the consistency (Seifriz, 1936) of the cytoplasm. The increased rate of phosphate leakage observed in 2 and 20 mM KH_2PO_4 solution may be due to the isotopic exchange facilitated by the high concentration of non-radioactive phosphate in the external solution.

Paper chromatographs (fig. 4) showed that after the three hour period of absorption, a large portion of the absorbed phosphate remained in the form of inorganic phosphate. Six other labelled unknown compounds were found. Radioisotope labelled phosphate compounds which leaked out from a deplasmolyzing rice root were mainly inorganic phosphate and four other unknowns. It was evident that solute leakage induced by deplasmolysis was not a result of complete disorganization of the proplasm or the membranes.

The degree of the damage that a rice root was subjected to during deplasmolysis was studied by observing the recovery of its phosphate absorption ability. One-gram samples of rice root were plasmolyzed in 0.4M mannitol solution for 1 hour, rinsed to remove the mannitol adhering on the root surface, and then transferred to 2 mM KH_2PO_4 solution containing radioactive phosphate. Different samples were taken from the absorption solution at given times, soaked with 4 changes of 200 ml of water within one hour to remove the phosphate which was not actively absorbed by the roots. The results (fig. 5) showed that after the first 30 minutes to an hour of immersion in the absorption solution, deplasmolyzing roots started to accumulate phosphate at a steady but lower rate than that of the normal rice root. This steady phase of absorption continued to the 12 th hour at the end of the experiment while the absorption of a normal root was a linear function of time for about 7 hours and continued to absorb phosphate at a declining rate from the 7 th to the 12 th hour.

Experimental evidence showed that phosphate exosmosis accompanied the deplasmolysis of the cells. It offers a possiblity of determining the osmotic pressure of plant tissues by detecting solute leakage when incipient plasmolysis can not be seen clearly in the plant tissues such as rice roots. Rice root samples prefed with radioactive phosphate were soaked in mannitol solutions of different concentration for one hour and then transferred to water. It was

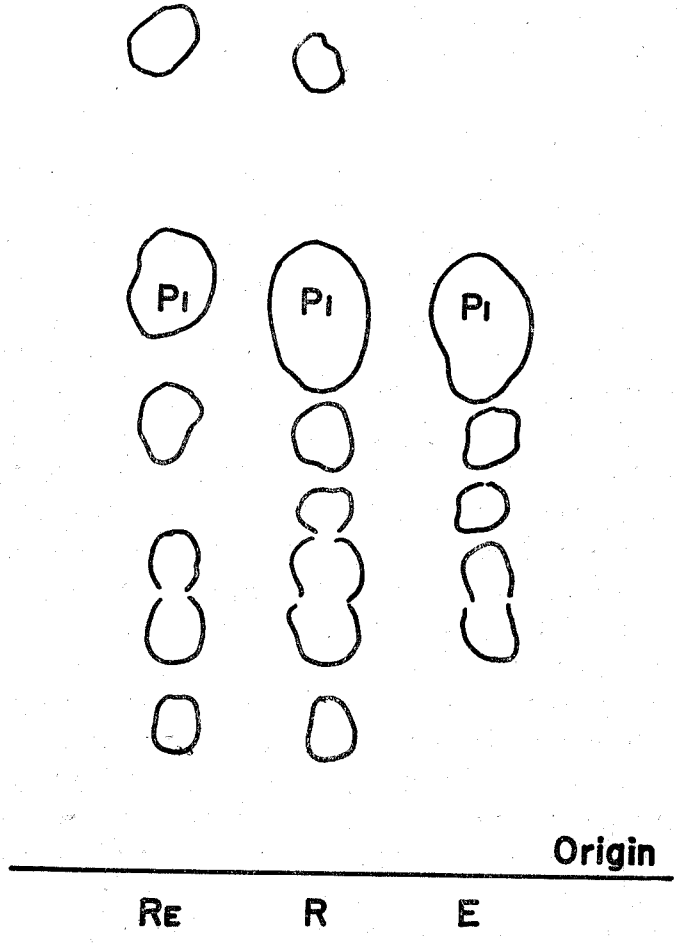

Fig. 4. Autoradiograph of a paper chromatograph of the P^{32}-labelled compounds in rice root and leakage. R and R_E, P^{32}-labelled compounds in root before and after the leakage induced by plasmolysis-deplasmolysis treatment. The roots were ground and extracted with 80% ethanol. E, P^{32}-labelled compounds in leakage. The leakage was evaporated to dryness and redissolved in 80% ethanol for paper chromatographing.

found that the roots which had been treated with mannitol solutions of the concentration higher than 0.22M lost appreciable amount of phosphate. The amount of phosphate loss increased sharply as the concentration of mannitol solution increased from 0.24 to 0.40M (fig. 6). The OP of the cell sap of the rice root was 0.23M as determined with a cryoscopic method. The value was very much the same as that indicated by the transition point of the curve in fig. 6.

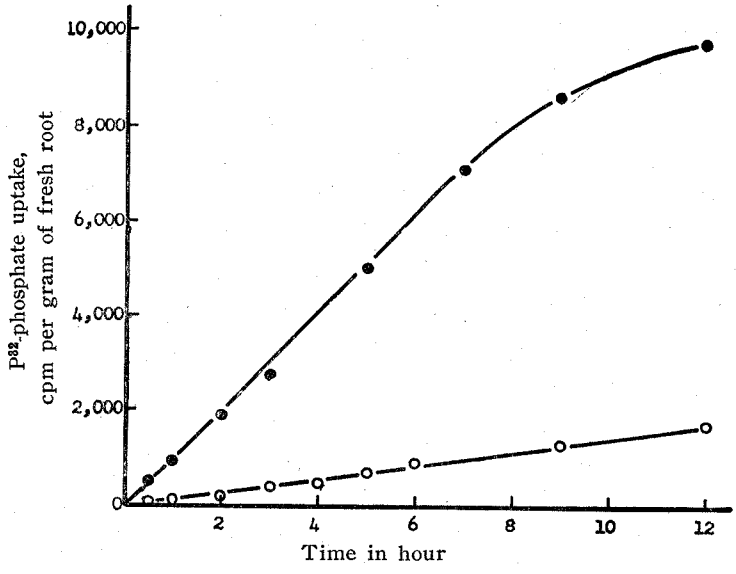

Fig. 5. The absorption of phosphate by untreated and plasmolyzed rice root. Rice roots were plasmolyzed in 0.4 M mannitol solution and then transferred to 2 mM KH_2PO_4 solution for absorption. Samples were rinsed in water for 1 hour after they were taken from the absorption solution to remove the diffusible phosphate in the outer space. Untreated rice roots were used as the control. ●, control; O, plasmolyzed roots.

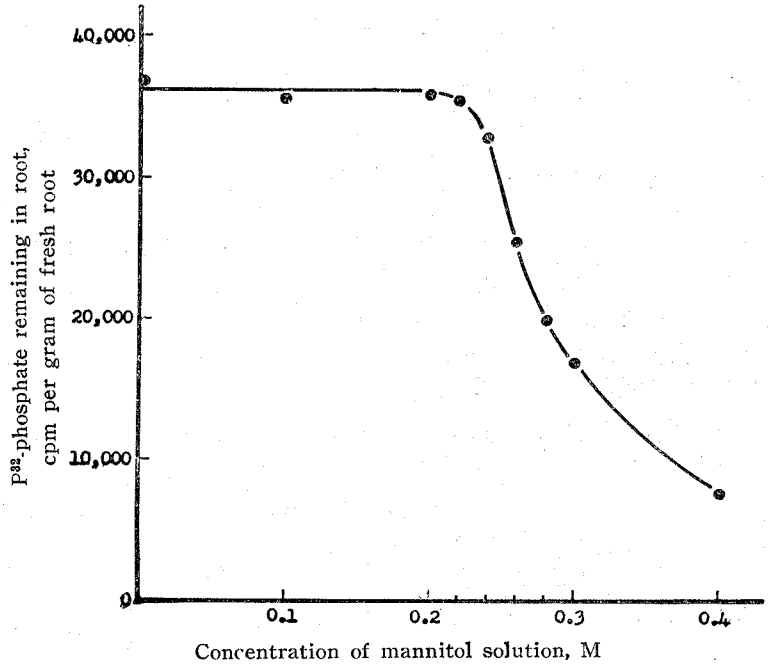

Fig. 6. The loss of absorbed phosphate from rice roots which had been immersed in mannitol solution of different concentrations for one hour before being transferred to water.

Summary

Excised rice roots accumulated phosphate as a linear function of time for seven hours and continued thereafter to absorb at a declining rate. Plasmolyzing rice roots absorbed phosphate actively as well as turgid roots. The absorption can be effectively suppressed with 2,4-dinitrophenol.

Large amount of phosphate exosmosis was induced by immersing plasmolyzed rice roots into solutions of low osmotic pressure or water. The amount and the rate of phosphate leakage were proportionately related to the rate of water entry into the root during deplasmolysis. This leakage was considered to be essentially a process of diffusion having a Q_{10} of 1.3 in the temperature range of 20–40°C.

Loss of the dry matter of the root always accompanied phosphate exosmosis.

After the period of exosmosis, a deplasmolyzed rice root reabsorbed and accumulated phosphate at a steady but lower rate than the normal root.

A new method for determining the osmotic pressure of plant tissues by detecting solute leakage was suggested.

溶液滲透壓對稻根之磷酸吸收與流失現象之影響

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切離之稻根吸收溶液中磷酸離子而蓄積於根內之量，在試驗開始七小時內，與時間成正比，七小時後至第十二小時間，吸收速度漸減。稻根在高壓溶液中，雖有脫水現象，仍保有吸收及蓄積磷酸之機能。此種蓄積作用與新陳代謝有關，可以2,4-二硝基酚（2,4-dinitrophenol）予以抑制。

當稻根被浸於純水或高壓溶液中，原蓄積於根內之磷酸有少量流失於根外。如先將根在高壓溶液中脫水，而後浸於純水或滲透壓較低之溶液中，根組織內之磷酸即大量流失於根外。其流失量或速度與外液滲壓轉變之大小有關。即脫水稻根之再吸水速度愈大，根內磷酸之流失愈多。控制外液滲透壓使緩慢降低，以減低脫水根之再吸水速度，則流失之磷酸量顯著減少。如將稻根先浸於水而後浸於高壓溶液中。無大量磷酸流失之現象。

磷酸流失現象可能屬擴散作用，在20–40°C之間，流失速度之溫度係數Q_{10}爲1.3。放射線自動映像（Autoradiograph）顯示，稻根吸收磷酸三小時後，根內之放射性磷，以無機磷酸爲多，並有六種含放射性磷之物質。由於脫水與再吸水而大量流失於根外者，亦以無機磷酸爲主，另有根內之四種含磷物質。

當脫水稻根再吸水之際，除根內之含磷化合物流失外，根之乾物質含量亦顯著減少。

脫水稻根經再吸水三十分鐘後，逐漸恢復其吸收及蓄積磷酸之機能。其吸收速度雖較正常根爲慢，但在試驗進行之十二小時內，一直保持平穩之蓄積速度。

因脫水與再吸水可引起根組織內溶質之大量流失，而提供一間接測定細胞液滲透壓之方法。

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