PHOSPHATE ABSORPTION AND LONG-DISTANCE TRANSPORT IN WHEAT SEEDLINGS

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Summary

The absorption of phosphate by the roots and simultaneous transport to the tops of intact wheat seedlings has been studied over the concentration range 0·0001–50 mM.

In the low concentration range (< 1 mM), phosphate absorption is characterized by the operation of two hyperbolic isotherms which represent sites of different affinity. Both sites may be located at the plasmalemma. Alternatively, one site may be at the plasmalemma, with the other representing phosphate absorption by the microbial population on the root surface. Irrespective of location, it is postulated that both sites are involved in absorption of the predominant $H_2PO_4^-$ ion species.

At high concentrations (> 1 mM) a linear absorption isotherm is obtained. This result is consistent with the involvement of a passive absorption component across the plasmalemma.

Long-distance transport of phosphate to the tops is described by a single hyperbolic isotherm at low concentrations, and a linear isotherm at high concentrations.

The simultaneous absorption and transport studies establish beyond doubt, that the two low concentration mechanisms of phosphate absorption do not correspond to system 1 and system 2 of the dual absorption mechanism.

I. Introduction

Examination of ion absorption by plant roots over a wide concentration range has demonstrated the existence of two different mechanisms for the absorption of a single ionic species (Epstein, Rains, and Elzam 1963; Elzam, Rains, and Epstein 1964; Jackman 1965; Epstein 1966). The absorption isotherm is characterized by a high affinity, low K_m system (system 1) operative at concentrations below 1 mM, and a low affinity, high K_m system (system 2) operative at higher concentrations. Recently, the hypothesis has been developed that system 1 mediates ion passage across the plasmalemma, while system 2 mediates ion movement across the tonoplast (Luttge and Laties 1966; Torii and Laties 1966). Ion transport to the tops of corn seedlings has the same characteristics as system 1 absorption (Luttge and Laties 1966), wherein the plasmalemma is the only membrane involved.

Although dual absorption mechanisms have been described for many inorganic ions (Epstein 1966) evidence for their involvement in phosphate absorption is limited

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to petiolar vascular bundles and storage tissues (Bieleski 1966). The absorption of phosphate by higher plants was considered to involve only the $H_2PO_4^-$ ion over the pH range 4·5–7·5 (Van den Honert 1937), until Hagen and Hopkins (1955) concluded from a kinetic analysis of phosphate absorption rates by excised barley roots, incubated in phosphate solutions ranging in concentration from 0·001 to 0·1 mM and at pH values ranging from 4·0 to 7·7, that both $H_2PO_4^-$ and HPO_4^{2-} are absorbed through sites of different affinity. Subsequent studies at pH 4·0, where 98·6% of the total phosphate is present as $H_2PO_4^-$, and only 0·06% present as HPO_4^{2-}, have likewise been interpreted to imply concomitant absorption of both ion species through separate sites (Hagen, Leggett, and Jackson 1957; Hagen and Hendricks 1958; Noggle and Fried 1960; Andrew 1966).

The above interpretation of the two apparent sites of phosphate absorption has recently been challenged. Carter and Lathwell (1967) suggested that dual mechanisms were operative in absorption by excised corn roots of $H_2PO_4^-$ only from KH_2PO_4 solutions ranging in concentration from 0·001 to 0·26 mM, and at pH 4·0. Edwards (1968) similarly suggested that absorption of $H_2PO_4^-$ ions by dual absorption mechanisms offered a far more plausible explanation of the two apparent sites than that based on involvement of both $H_2PO_4^-$ and HPO_4^{2-} ion species. However, development of these ideas demands further consideration of the location and nature of the rate-limiting steps in phosphate absorption.

The present paper examines the mechanisms involved in phosphate absorption and long-distance transport to the tops of seedlings of *Triticum vulgare* cv. Gabo. Phosphate concentrations ranging from 0·0001 to 50 mM were employed in the present study. This concentration range is the one usually employed in studies of dual absorption mechanisms, and is much wider than the 0·001–1 mM range within which phosphate absorption was previously studied (Hagen and Hopkins 1955; Hagen and Hendricks 1958; Noggle and Fried 1960; Carter and Lathwell 1967).

II. Materials and Methods

Seeds of *T. vulgare* cv. Gabo were placed in aerated, de-ionized water for 24 hr. The germinated seeds were then planted between two layers of cheesecloth on screens suspended over an aerated nutrient solution of the following composition (μM): K 255; Ca 250; Mg 100; NH_4 100; NO_3 750; SO_4 100; Cl 100; H_2PO_4 5; B 3; Fe 2 [as sodium ferric ethylenediamine di(*o*-hydroxyphenylacetate)]; Mn 1; Zn 0·5; Cu 0·1; Co 0·04; and Mo 0·02. The pH of the solution, which drifted to as low as 5·0, was controlled to 5·6 twice daily, while the temperature of the nutrient solution was maintained at 20±1°C. The seedlings were grown in a glasshouse. Three days after planting, the top layer of cheesecloth was removed, and the nutrient solution was totally renewed.

When 6 days old, individual seedlings were selected and placed on stainless steel screens over 1-litre plastic beakers. These seedlings (30 per screen) were maintained in 0·6 mM $CaSO_4$ for about 30 min, until just prior to the commencement of the absorption period.

Absorption and transport of phosphate were followed over the concentration range 0·0001–50 mM. All experimental solutions contained ^{32}P-labelled KH_2PO_4 and 0·6 mM $CaSO_4$ and were adjusted to pH 5·6. At this pH, 97·5% of the total phosphate is present as $H_2PO_4^-$ and 2·5% is present as HPO_4^{2-}. The solutions were continuously aerated, and maintained at a temperature of 24°C. Samples of 10 seedlings were withdrawn at 1, 2, and 3 hr. The roots were rinsed in distilled water for 1 min, prior to separation of the seedlings into roots, tops, and seeds. After obtaining fresh weights, the plant parts were packed into planchets and dried under an infrared lamp. The plant samples were assayed for radioactivity with a Geiger–Müller tube, along with

appropriate standards from the experimental solutions. From the known specific activity of the particular phosphate solution, the amounts of phosphate absorbed by the roots and transported to the tops (μmoles/g fresh weight of roots) were calculated.

III. Results

The isotherm for phosphate absorption by intact wheat seedlings from phosphate solutions in the concentration range 0·0001 mM–50 mM is characterized by the involvement of three distinct absorption mechanisms (Fig. 1). In the low concentration range (0·0001–1·0 mM) the absorption isotherm consists of two hyperbolic absorption mechanisms. The first reaches saturation at about 0·07 mM total phosphate concentration, while the second approaches saturation at a concentration of the order of 1·0 mM. As the phosphate concentration is increased to within the high concentration range (1·0–50 mM), phosphate absorption increases linearly with increase in phosphate concentration.

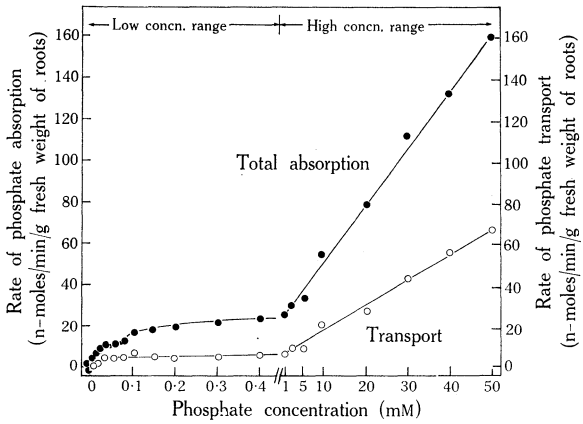


Fig. 1.—Isotherms for the absorption and long-distance transport of phosphate by intact wheat seedlings over the concentration range 0·0001–50 mM.

At all concentrations of phosphate studied, total phosphate absorption by the intact wheat seedlings remained essentially linear over the 3 hr duration of the experiment [Fig. 2(*a*) shows data for phosphate concentrations from 0·0001 to 1·0 mM]. The maintenance of such steady-state root absorption satisfies the requirement for analysis of the data by Michaelis–Menten kinetics (Michaelis and Menten 1913; Epstein and Hagen 1952).

By contrast with the isotherm for phosphate absorption, the isotherm for long-distance transport of phosphate to the plant tops indicates that only one hyperbolic transport mechanism is operative in the low concentration range (Fig. 1). However, the rate of transport of phosphate to the tops increases linearly with concentration in the high concentration range (Fig. 1).

Transport of ^{32}P-labelled phosphate to the tops of seedlings exposed to phosphate solutions in the low concentration range is characterized by an approximately exponential increase in the amount transported with increased time of incubation of the seedlings in the labelled solutions [Fig. 2(*b*)]. Thus, at no stage are steady-state

transport conditions obtained in this low concentration range. The rates of phosphate transport (Fig. 1) are calculated from the increase in ^{32}P activity of the tops between 2 and 3 hr. This method is considered to give the best approximation towards a steady-state system. In fact, the pattern of response of phosphate transport to increasing concentration, within the low concentration range, was very similar irrespective of whether the rates were computed over the 0–1, 1–2, or 2–3-hr intervals; the isotherms essentially exhibited a vertical displacement over the different time intervals. Recently, Crossett (1968) has shown that translocation of ^{32}P-labelled phosphate to the tops of intact barley seedlings incubated in 0·001 mM KH_2PO_4 attained a stable rate only after 6 hr of absorption. This observation justifies the use of the transport rate calculated between 2 and 3 hr as the best approach to steady-state conditions. By contrast, phosphate transport rates to the tops of seedlings incubated in phosphate solutions within the high concentration range were essentially linear over the full 3 hr duration of the experiment.

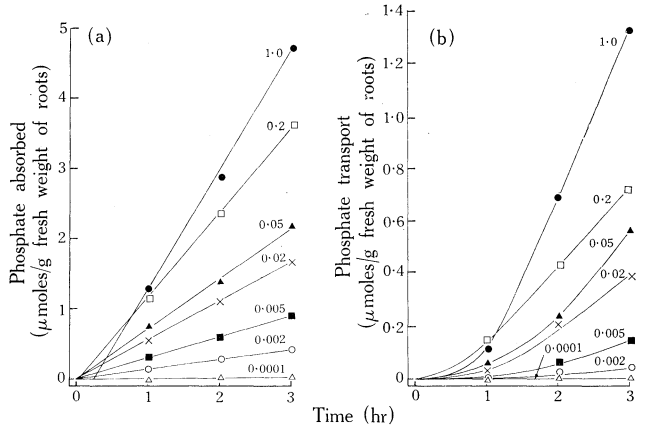


Fig. 2.—Time course of phosphate absorption (*a*) and transport (*b*) by wheat seedlings at the phosphate concentrations (mM) indicated.

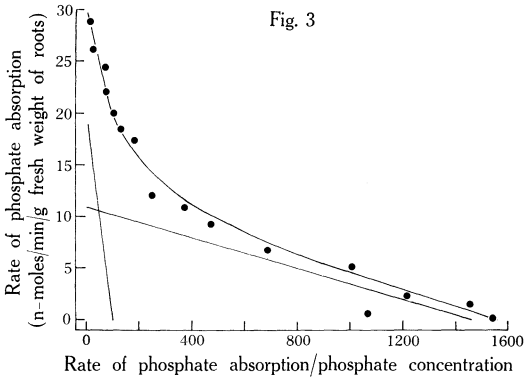
Kinetic Analysis

Kinetic analysis of both the phosphate absorption and transport data was used to resolve more fully the mechanisms involved, particularly with reference to the nature and location of the rate-limiting steps.

A curvilinear relationship was obtained on plotting the rate of total phosphate absorption as a function of the rate of absorption/phosphate concentration (Eadie 1942) over the concentration range from 0·0001 to 1·0 mM (Fig. 3). The curve was resolved into two linear components. Although these absorption mechanisms possess maximum absorption rates of similar magnitude, they differ quite considerably in affinity (Table 1).

Eadie plots were also made of phosphate transport to the tops of the wheat seedlings over the concentration range 0·0001–0·4 mM using the amount of phosphate transported to the tops between 2 and 3 hr as the best approach to a steady-state transport system. By contrast with absorption, only one transport mechanism is

operative over this concentration range; a linear relationship was obtained for all concentrations greater than 0·005 mM (Fig. 4). Because of the departure from strict steady-state conditions, the kinetic constants obtained for transport at phosphate concentrations less then 1 mM (Table 1) are apparent values which deviate considerably from the real values consistent with the operation of a steady-state transport system. At low concentrations of phosphate ($< 0{\cdot}002$ mM), the relation between rate of absorption and rate of absorption/phosphate concentration departs markedly from linearity (Fig. 4). This discrepancy is due to a decrease in the response of transport rates to concentration at the lowest phosphate concentrations. This lag phase is almost certainly associated with initial transport of unlabelled endogenous phosphate. As absorption proceeds, the specific activity of the phosphate which is transported builds up to a constant value. At this point, linear transport of ^{32}P-labelled phosphate is obtained (Crossett 1968).



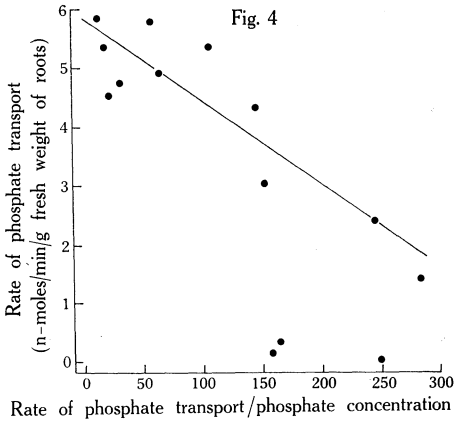


Fig. 3.—Eadie plot of phosphate absorption by intact wheat seedlings over the concentration range 0·0001–1·0 mM.

Fig. 4.—Eadie plot of phosphate transport to the tops of wheat seedlings over the concentration range 0·0001–0·4 mM.

At high phosphate concentrations (> 1 mM) both the rate of absorption and transport increase linearly with increasing phosphate concentration (Fig. 1), indicating that both processes are diffusion controlled (Torii and Laties 1966). No kinetic constants can thus be evaluated for either phosphate absorption or transport in the high concentration range. However, an improved estimate of the kinetic constants of the low concentration transport mechanism was obtained by consideration of the data at concentrations of 1, 2, and 5 mM; there is a suggestion (Fig. 1) that this mechanism saturates at a concentration of 2–5 mM phosphate. In addition, transport was approximately a linear function of time at these concentrations. Accordingly, the apparent K_m of the low concentration transport mechanism was estimated to be about 9×10^{-5}M, while the maximum transport rate (V_{max}) was 11·2 n-moles/min/g fresh weight of roots (Table 1). This value of V_{max} is considerably higher than the value obtained under non-steady-state conditions, but is still much less than the maximum rate of root absorption obtained at low concentrations (< 1 mM).

The K_m of the transport process, estimated assuming saturation at 2–5 mM phosphate, is closely similar to the K_m of the absorption mechanism 1B (Table 1). It is suggested, therefore, that these represent one and the same process, viz. the mechanism which controls the rate of phosphate absorption at the plasmalemma.

TABLE 1

APPARENT K_m AND V_{max} VALUES FOR TOTAL PHOSPHATE ABSORPTION AND TRANSPORT IN INTACT WHEAT SEEDLINGS

Wheat seedlings were incubated in KH_2PO_4 solutions ranging in concentration from 0·0001 to 50 mM. pH was 5·6

Concentration Range	Absorption K_m (M)	Absorption V_{max} (n-moles/min/ g fresh wt. of roots)	Transport K_m (M)	Transport V_{max} (n-moles/min/ g fresh wt. of roots)
Low (0·0001–1·0 mM)				
Mechanism 1A	$7·4 \times 10^{-6}$	10·9	—	—
Mechanism 1B	$2·0 \times 10^{-4}$	18·7	$1·4 \times 10^{-5}$*	5·9*
			$9·2 \times 10^{-5}$†	11·2†
High (1·0–50 mM)				
Mechanism 2	—‡	—‡	—‡	—‡

* Kinetic constants obtained by analysis of transport data under non-steady-state conditions.

† Kinetic constants obtained by analysis of transport data under steady-state conditions, using three concentrations only, viz. 1, 2, and 5 mM.

‡ No kinetic constants could be obtained for the passive processes operative at high concentrations of phosphate.

IV. DISCUSSION

Kinetic analysis of the rates of phosphate absorption by the roots of intact wheat seedlings has led to the resolution and characterization of three distinct absorption mechanisms (Fig. 1; Table 1). The two low concentration mechanisms correspond with those previously described for phosphate absorption at concentrations less than 1 mM (Hagen and Hopkins 1955; Hagen, Leggett, and Jackson 1957; Hagen and Hendricks 1958; Noggle and Fried 1960; Leggett, Galloway, and Gauch 1965; Andrew 1966; Carter and Lathwell 1967; Edwards 1968). However, the third mechanism, which shows a linear response to increasing phosphate concentration, is only observed at concentrations greater than 1 mM.

As a result of experiments with vacuolated and non-vacuolated corn root cells, Torii and Laties (1966) concluded that the low concentration system 1 ion absorption is mediated across the plasmalemma. Whereas K^+, Rb^+, and Cl^- show the saturation kinetics of a single system at concentrations less than 1 mM (Elzam and Epstein 1965; Epstein, Rains, and Elzam 1965; Jackman 1965; Luttge and Laties 1966; Torii and Laties 1966), phosphate absorption is characterized by the operation of two separate systems of differing affinity. Certainly, one of these mechanisms, viz. 1B (Table 1), must be located at the plasmalemma. The other low concentration absorption mechanism 1A may also be located at this membrane. If so, both are postulated as being involved in absorption of the predominant $H_2PO_4^-$ species.

Alternatively, while one site (mechanism 1B) is located at the plasmalemma, the other (mechanism 1A) may represent phosphate absorption by the microbial population which was undoubtedly present on the root surface of these plants, which were raised under non-sterile conditions (Rovira 1965). The K_m for phosphate absorption by mechanism 1A corresponds closely with that for the high affinity mechanism of phosphate absorption by yeast (Leggett 1961) and by excised mycorrhizas of *Pinus radiata* (Bowen and Theodoru 1967). Further support for the involvement of microorganisms in phosphate absorption by plant roots is afforded by the studies of Bowen and Rovira (1966) and Barber and Loughman (1967), who both compared phosphate uptake by sterile and non-sterile root systems at a limited number of low phosphate concentrations. Both schools obtained marked effects of microorganisms on phosphate absorption by plant roots. However, complete resolution of the low concentration phosphate absorption mechanisms, particularly in regard to the role of microorganisms, demands determination of the isotherms using plants cultured under sterile conditions.

The linear absorption isotherm obtained for phosphate at concentrations greater than 1 mM (Fig. 1) contrasts markedly with the basically hyperbolic isotherm with several distinct heterogeneities described for Cl^- (Elzam, Rains, and Epstein 1964) and K^+ (Epstein 1966) absorption by excised barley roots. However, it resembles the linear to exponentially rising isotherms for Cl^- and Rb^+ absorption into non-vacuolate corn root tip cells at salt concentrations ranging from 1 to 50 mM which were obtained by Torii and Laties (1966) and interpreted by them as diffusive breakthrough across the plasmalemma. Certainly, the present results for phosphate in the high concentration range are consistent with the involvement of a passive component of absorption across the plasmalemma.

The question now arises as to why phosphate absorption, at concentrations greater than 1 mM, does not show the characteristics of mechanism 2 ion absorption. In vacuolate cells, Torii and Laties (1966) considered that rapid diffusion across the plasmalemma overcomes the rate limitation set by saturation of mechanism 1, and allows mechanism 2, assumed to reside in the tonoplast, to become rate limiting. If Torii and Laties' hypothesis is accepted, the failure of phosphate absorption to show the above characteristics may be interpreted to indicate that passage of phosphate across the tonoplast does not become rate limiting at high concentrations. This may be related to the rapid incorporation of a substantial fraction of the absorbed phosphate into organic compounds within a very short time interval (Loughman and Russell 1957; Jackson and Hagen 1960). The location of this organic pool within the cytoplasm would prevent the rapid build-up in inorganic phosphate concentration necessary for the tonoplast to become a rate-limiting membrane. Recently, however, Welch and Epstein (1968, 1969) have provided convincing evidence that both mechanisms 1 and 2 of K^+ and Cl^- absorption operate in parallel across the plasmalemma. In addition, they were unable to find any evidence for passive permeation of the plasmalemma by the above ions. This situation does not necessarily hold for phosphate; in fact, present results indicate phosphate is passively absorbed across the plasmalemma at high concentrations.

The obvious conclusion that may be drawn is that there is no observable metabolically mediated mechanism 2 of phosphate absorption at high concentrations.

This conclusion is thus independent of the controversy regarding whether mechanism 2 is located at the plasmalemma (Welch and Epstein 1968, 1969) or at the tonoplast (Torii and Laties 1966).

The long-distance transport of phosphate to the tops of wheat seedlings is characterized by a single hyperbolic isotherm in the low concentration range and a linear isotherm in the high concentration range (Fig. 1). These transport features are similar to those of K^+, Rb^+, and Cl^- to the tops of young corn seedlings (Luttge and Laties 1966). However, in attempting to interpret the present data, a central issue concerns the form in which phosphate is transported. Firstly, a considerable part, at least, of the absorbed phosphate is rapidly metabolized and incorporated into organic compounds (Loughman and Russell 1957; Jackson and Hagen 1960). Secondly, phosphate is transported to plant tops in the inorganic form (Loughman and Russell 1957). Thirdly, notwithstanding the conclusions of Loughman (1966), unequivocal evidence that phosphate transport to plant tops is directly dependent on prior incorporation into organic compounds is lacking. Phosphate transported to the plant tops may either pass through a small, restricted fraction of the organic pool (Crossett 1968), or even by-pass the organic pool altogether. This suggestion is strengthened by the close correspondence between the K_m values for phosphate absorption (mechanism 1B) and transport at low concentrations. Thus, at low concentrations both phosphate absorption and transport are mediated by mechanism 1, operative at the plasmalemma. At high concentrations (1–50 mM), the linearity of both the absorption and transport isotherms indicates that phosphate ions are moved passively across the plasmalemma.

The simultaneous studies of phosphate absorption and long-distance transport in intact wheat seedlings have demonstrated conclusively that the two low concentration phosphate absorption mechanisms observed in this and in previous studies (Hagen and Hopkins 1955; Hagen, Leggett, and Jackson 1957; Hagen and Hendricks 1958; Noggle and Fried 1960; Leggett, Galloway, and Gauch 1965; Andrew 1966; Carter and Lathwell 1967; Edwards 1968) do not correspond to system 1 and system 2 of the dual absorption mechanism for phosphate as previously suggested (Carter and Lathwell 1967; Edwards 1968). Nevertheless, recent criticism of the interpretation of these two mechanisms in terms of both $H_2PO_4^-$ and HPO_4^{2-} ion species (Hagen and Hopkins 1955; Hagen, Leggett, and Jackson 1957; Hagen and Hendricks 1958) is still valid. At pH values as low as 4·0, $H_2PO_4^-$ is the only species of any consequence present (Carter and Lathwell 1967). Although further resolution of the mechanism of phosphate absorption by plant roots awaits the use of sterile plant systems, it is considered that the two alternatives discussed in this paper, both involving absorption of $H_2PO_4^-$ alone, offer a far more logical hypothesis than that involving concomitant absorption of both $H_2PO_4^-$ and HPO_4^{2-} ion species.

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