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DIFFUSION OF PHOSPHATE TO PLANT ROOTS IN SOIL

I. QUANTITATIVE AUTORADIOGRAPHY OF THE DEPLETION ZONE

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SUMMARY

Improved resolution in autoradiography, achieved by the use of the low ß energy isotope, P^{33}, as tracer for soil phosphorus, enables the exchangeable phosphorus in a soil block to be measured quantitatively. A technique is described for the autoradiography of the P-depletion zone around the roots growing in soil, from which the P gradients are measured by microdensitometry.

The amounts of P taken up by rape (*Brassica napus*) on a P-treated Begbroke Sandy Loam compared well with that removed from the soil as measured from the autoradiograph of the depletion zone. The P gradient around the roots suggests intense root hair activity; but the zone of depletion extended well beyond the tips of root hairs. The experimentally observed gradient is much closer to the one predicted from diffusion theory considering uniform depletion from within the equivalent root hair cylinder, than to the one obtained assuming the root hairs are inactive.

A rapid depletion of up to about 60 per cent of the exchangeable P was observed within the root hair cylinder during the initial 3 days of absorption. The corresponding concentration of P in solution within the cylinder determined from a desorption isotherm, is hence brought down to a low level very rapidly, and is held at or near this level at later periods. The amounts transferred into the root hair cylinder from outside as calculated from a diffusion model were lower than the experimental values. It is suggested that the discrepancy may lie in the calculation of the effective diffusion coefficients for P in the soil from a P-desorption isotherm, owing to difficulties involved in simulating the root environment in the desorption isotherm experiment.

INTRODUCTION

The possibilities of predicting the uptake of potassium and phosphorus by plants from a knowledge of the diffusible amounts of these elements in the soil, their concentration dependent diffusion coefficients and the characteristics of the root system, have been shown by Drew, Vaidyanathan and Nye[4 5 6] who made use of the model proposed by Nye[9] for plants with or without root hairs. In these earlier studies, use was made of the experimentally observed uptakes in calculating the average concentrations of these elements at the surface of the root or of the 'equivalent root hair cylinder' (see Nye[9]). Olsen and Watanabe[11] used a value for the root surface concentration estimated from uptake rates measured over periods upto 24 hours and obtained as the midpoint of two assumed boundary conditions. These studies suggest the need for a method for the direct *in situ* measurement of the P gradients around the roots growing in a soil.

This paper reports an experiment made with rape (*Brassica napus*) seedlings, with a well-developed root hair system, grown in a phosphorus treated soil. The phosphate gradient around the roots and the amounts of P taken up from within the root hair cylinder were measured by quantitative autoradiography of the depletion zone. Depletion from outside the root hair cylinder, calculated from diffusion theory is compared with the experimentally determined value at different portions of the root system.

In most of the previous autoradiographic work to demonstrate P depletions around plant roots[7 13], P^{32} has been used as the tracer. The maximum energy of this isotope being high (1.71 Mev), it is not possible to obtain a satisfactory resolution for quantitative evaluation of depletions. The isotope P^{33}, which has a much lower beta energy ($E_{max.} = 0.25$ Mev), was used in the present work. Although improved resolution with P^{33} was reported as early as in 1956[8], this isotope has not been used to any considerable extent as tracer, especially in studies on soil phosphorus.

EXPERIMENTAL

Treatment and preparation of soil

The soil used in this experiment was a Begbroke Sandy Loam (pH in 0.01 *M* $Ca(NO_3)_2$: 6.9; total P: 37.7 $\times$ 10^{-6} moles per g), ground and passed through a 100 mesh sieve (0.152 mm). Ten microcuries of $H_3P^{33}O_4$ with 5 $\times$ 10^{-6} moles of P as Ca $(H_2PO_4)_2$ were added to each gram of soil with the minimum amount of water required to handle the soil conveniently for mixing and packing. Uniform labelling of the soil was achieved after several hours of thorough mixing by hand with a spatula.

The labelled soil was packed at a bulk density of 1.1 air dry g ml^{-1}, into a 9 cm long, 2 cm wide, and 0.5 cm thick block in a perspex cell, the front of which was made up of a transparent detachable plate. The cell containing the soil block was mounted on a porous PVC membrane with a 30-cm water column attached to it from below. This resulted in a final volumetric moisture content of 0.41 in the soil block, which was maintained constant throughout the experiment.

Based on the specific activity of the soil solution on the 7th day after labelling and P treatment, the exchangeable P content of the soil was 7.8×10^{-6} moles ml^{-1}. The long-term exchangeable P as obtained from the specific activity of phosphorus extracted from the soil after 70 days from labelling, in 48 hours by an anion-exchange resin was 13.5×10^{-6} moles ml^{-1}.

Plant growth details

Immediately after preparation, the soil block with the water column was transferred to a growth chamber maintained at 25°C. Seven days after labelling (which was adequate for reasons discussed later) two germinated rape seeds were sown on the top of the soil block against the inner surface of the front plate of the cell and the cell was tilted so as to induce the roots to grow down along the surface of the block. The light intensity was maintained at 3000 foot candles and the tops were allowed to grow into a closed perspex chamber in order to reduce transpiration losses of water to a minimum. The amount of water moved into the soil during the growth of plants, as measured by the level of water in the column, was negligible. Observations were made on the lengths of roots every day.

The experiment was carried out on 3 replicate soil blocks.

Autoradiography

After 7 days of plant growth, when the primary roots almost reached the bottom of the block, the cells were detached from the water column for autoradiography. The front plate of each cell was removed and the surface covered with a 3 micron thick mylar film before bringing it into close contact with Kodirex X-ray film. After 4 hours' exposure, the film was developed, washed and dried.

Separate autoradiographic standards were prepared by diluting the same amount of the initially P^{33}-labelled soil with increasing amounts of unlabelled, P^{31}-treated soil, holding them at the same moisture level and under the same conditions as the experimental soil and exposing them for the same period.

The autoradiographs were then scanned through a Wooster Mark III recording microdensitometer. Densities were measured across the block every 0.5 cm along the surface starting from 0.2 cm from the top and also across the laterals. The centre of the root could be located on the scans and the densities at different distances away from the root surface read off from the scans. The densities were converted into concentrations by reference to the standards.

In order to examine the horizontal cross section of the depletion zones, one of the replicate blocks was frozen in liquid nitrogen at the end of the experiment, cut across at different heights in a cryostatic microtome and the transverse sections of the block exposed for autoradiography.

Soil and plant analyses and root measurements

The shoots of plants were harvested, dried and analysed for the total amount of P^{33} taken up by wet ashing and planchet counting. The total P^{33} added to the soil was also measured in the perchloric acid digest of a known amount of the initially labelled soil by planchet counting.

The soil block was immersed in water to wash the soil out of the root taking care to retain as many of the root hairs as possible intact. Observations on the roots were made under the microscope, at the same points as the ones where the autoradiographs were scanned. The diameter of the central root, lengths of root hairs and their numbers per unit root length were measured using a graduated eye piece graticule.

Desorption isotherm

Weights of soil labelled with P^{32}, but otherwise identical with the experimental block, corresponding to 1 ml of the block, were suspended in volumes varying from 2 ml to 5 l of 0.01 M Ca $(NO_3)_2$ solution, the pH of which was

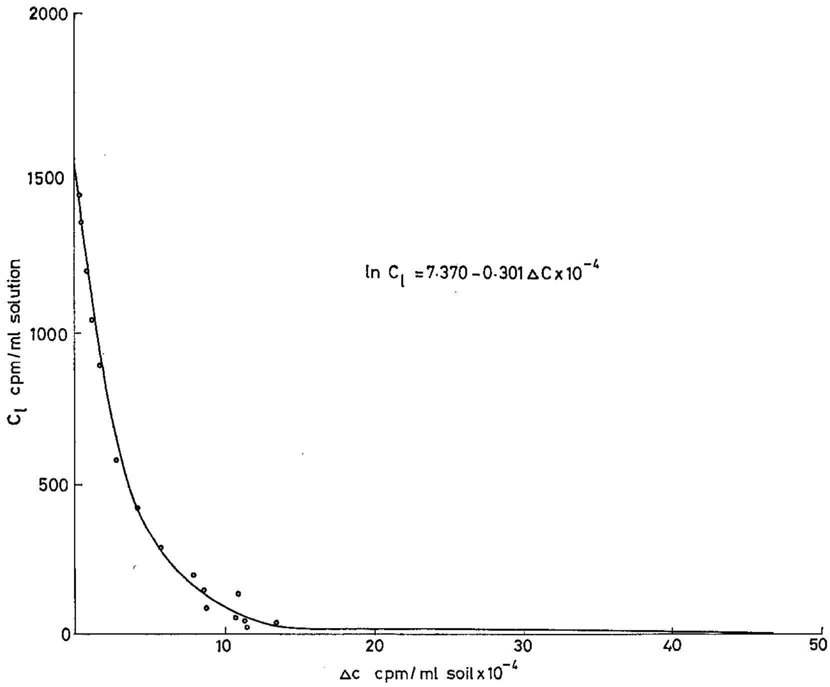

Fig. 1. Phosphate desorption isotherm. Concentration of P in solution (C_l) at varying levels of desorption of exchangeable P (ΔC).

previously adjusted to the same as that of the soil solution, and shaken by passing a stream of air through them for 24 hours at 25°C. The suspensions were centrifuged at 3000 rpm for 20 minutes, filtered and the filtrates analysed for P^{32} by Cerenkov counting.

From the experimentally observed values, the desorption isotherm was reconstructed to the same total no. of counts per unit volume of initial soil as in the soil blocks labelled with P^{33} for autoradiography. The relationship between ΔC, the change in cpm of exhangeable P per ml soil and C_l, the concentration of P in solution in cpm per ml, presented in Fig. 1 was such that

$$\ln C_l = 7.370 - 0.301 \Delta C \times 10^{-4} \quad (1)$$

The coefficient of correlation between ΔC and ln C_l being highly significant ($r = -0.974$), C_l at any given value of ΔC could be read out from the isotherm. C_{l_i}, the initial soil solution concentration in the undisturbed block, was 1561 cpm per ml.

The desorption isotherm was used for calculating the effective diffusion coefficients from the relation[9]:

$$(D) \simeq D_l v_l f_l \frac{\Delta C_l}{\Delta C} \quad (2)$$

where

(D) is the effective diffusion coefficient in $cm^2 \, sec^{-1}$
D_l is the diffusion coefficient of H_2PO_4 in free solution ($= 0.89 \times 10^{-5}$ $cm^2 \, sec^{-1}$)
v_l is the volumetric moisture content ($= 0.41$)
f_l is the impedance factor measured in a separate experiment ($= 0.37$)
C_l is the concentration of P in solution in cpm ml^{-1}

and

C is the total concentration of exchangeable P in the soil in cpm ml^{-1}.

RESULTS AND DISCUSSION

Quantities of phosphorus are presented in this paper as cpm of P^{33}. Approximate conversions to P^{31} may readily be made from the specific activity of the exchangeable P (measured at the time of sowing): 60 cpm = 10^{-9} moles.

Autoradiographs

The range of P^{33} in an absorber material, calculated as 0.37 $E_{max}^{1.5}$ [14], is 47 mg cm^{-2}, compared to 828 mg cm^{-2} for P^{32}. In the present experiment, the maximum depth of soil from which β particles can emerge was 0.03 cm, whereas under the same conditions, with P^{32} this depth would be about 0.53 cm.

Estimates made from the autoradiographs of standards showed a resolution as defined by Doniach and Pelc [3] (distance at which the grain density is one half of that observed directly over the source) of the order of 0.025 cm under the conditions used for preparing the autoradiographs and for scanning. Further improvement on this resolution is perhaps possible by using a film, thinner than 3μm, to separate the soil block from the X-ray film during exposure; by using X-ray films of finer grain density; and by using a smaller densitometer aperture in scanning. Although with the scan magnification used (× 5), it was possible to read off concentrations at points 0.01 cm apart, average concentrations measured over successive 0.05 cm thicknesses from the root surface were used in the calculation of depletions.

The scans showed clearly that the depletion zones extended well beyond the limits estimated by direct observations on the film with the naked eye, and any such attempts would obviously lead to erroneous conclusions.

Autoradiographs of the 3 replicates are presented in Plate 1. In block I the roots of the two plants grew separately whereas blocks II and III show marked overlaps of depletion zones. The results from block I alone are discussed in this paper. The autoradiographs of transverse sections of block III showed clearly the semicylindrical geometry of the depletion zones.

Examples of typical densitometer scans across block I are shown in Fig. 2. Scans of the standards prepared over a range of concen-

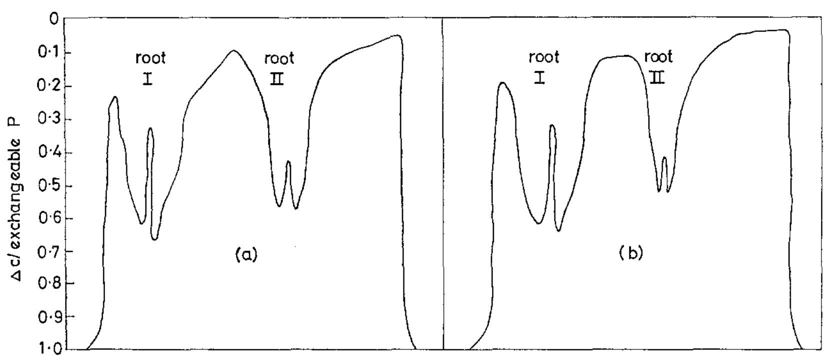

Fig. 2. Scans across the autoradiograph of block I at (**a**) 0.7 cm and (**b**) 2.2 cm from the surface of the block.

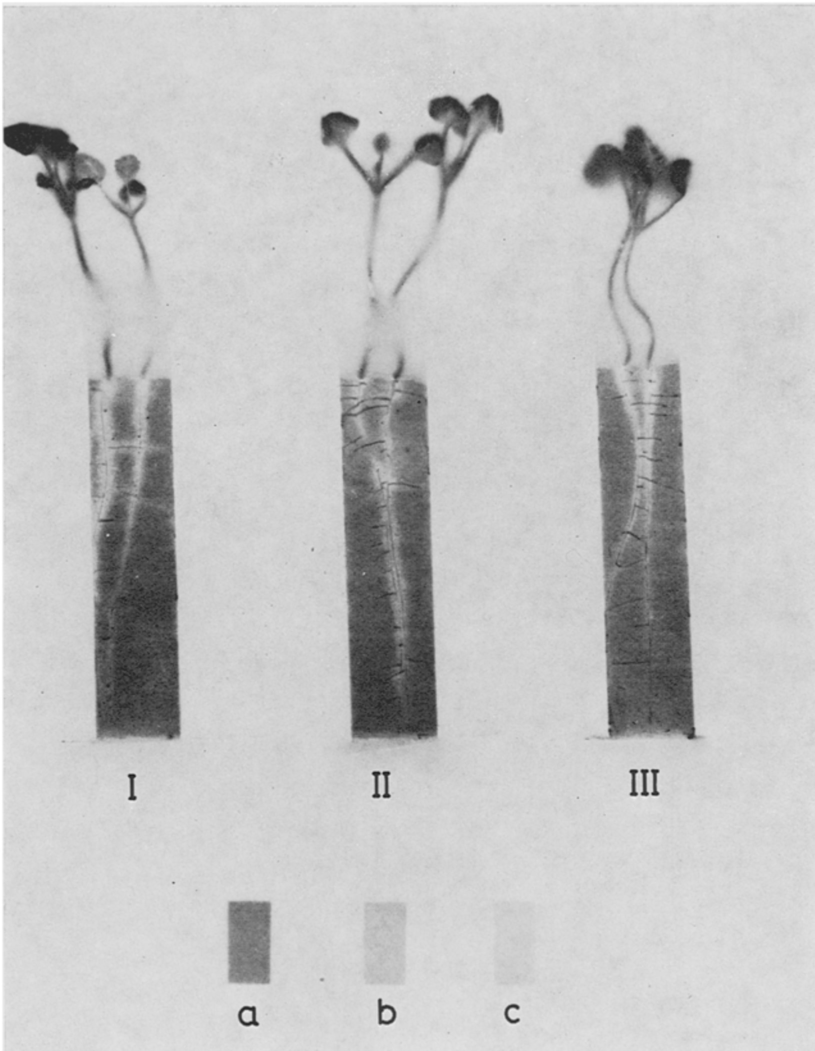

Plate 1. Autoradiographs of the replicate soil blocks I, II, and III after 7 days of plant growth. Results discussed in this paper are those from the two plants (plant I left and plant II right) on block I.
a, b and c show the densities corresponding to dilutions of 2, 5 and 10 times respectively, of the soil in the blocks with unlabelled soil.

trations showed a linear relationship between activity and film density for periods of exposure upto 4 hours.

Root measurements and plant analysis

The rate of elongation of the primary roots during the 7 day period of growth was steady, at about 1.25 cm per day. The radius of the primary root, that of the root hair cylinder and the number of root

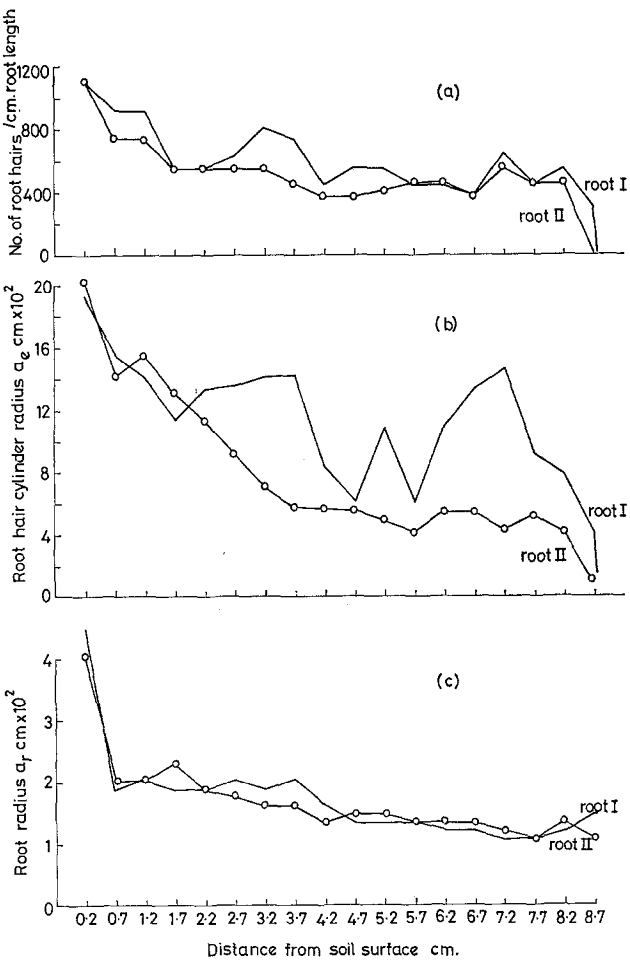

Fig. 3. Root measurements. Root hair density (**a**) and radii of the two primary roots (**c**) and of the equivalent root hair cylinders (**b**) at the points of scanning the autoradiograph.

hairs per unit root length, observed at the points of scanning, are presented in Fig. 3. The results presented in this figure are those observed for the primary roots of both plants growing in replicate number I.

Drew and Nye[4] observed that the root hairs of rye-grass grow normal to the root surface. Direct microscopical examination of the intact root system in this study showed the same tendency for the rape root hairs as well, although it was not possible to make measurements of their lengths and frequencies with satisfactory accuracy without washing the soil off. The root hairs on the extracted roots apparently retained their original bends and assuming there was no change in their overall lengths, the straight distances from the surface of the root to the tips of the majority of the root hairs without stretching them was added to the radius of the root at the point of observation to obtain the radius of the root hair cylinder.

Although the dimensions of the root hair cylinder over root lengths of comparable ages varied considerably from one plant to the other, it is possible to draw certain general conclusions from these measurements: (i) On both the plants, a tuft of dense and long root hairs was observed over a few millimeters from the base of the plant, where the central root itself was considerably thicker than at the younger regions. (ii) The root hairs grow rapidly at first, after which their rate of growth is much slower, so that the decrease in the radius of the cylinder down the root, below the region referred to in (i) above, is only slight, except for a few local variations and for a short length at the root tip. (iii) Under identical conditions of growth, the root system of one plant may be considerably different from that of another. The root hair cylinder for plant I had a smaller radius than that of plant II in this experiment. The width of the depletion zone and the amounts of P taken up varied accordingly.

Although several laterals appeared during the experiment, their contribution to absorption in the present experiment was only a fraction of the total. Scans across the 12 laterals of varying lengths and ages (maximum age: 4 days) that appeared on the surface of the block showed depletion zones only around 3 of those containing portions more than 3 days old. Depletions around the rest of them were negligible, in contrast to the depletions observed around the primary root which were detected around surfaces more than $1\frac{1}{2}$ days old (see Fig. 5). A few laterals had grown into the soil block and although it was not possible to measure the depletions around them, it could reasonably be concluded that the contributions from them during the 7-day growth in this experiment, was only slight.

The total amount of P found in the aerial parts of the two plants was 369474 cpm. It was not possible to measure the P contained in the roots; but from other experiments with rape grown on the same soil, this was estimated to be less than a fifth of the total amount in the whole plant at the same stage of growth as in this experiment.

Depletions from soil

For calculating the total amount of P taken up at any point on the root in the experiment, the zone of depletion around it was considered as made up of a series of successive concentric hollow semicylinders, 0.5 cm high and 0.05 cm thick, the inner radius of the smallest of them being the radius of the central root itself and the outer radius of the largest being that of the total depletion zone. The depletion from each semicylinder was obtained as the product of its volume and the corresponding decrease in concentration read off from the scans.

The total amounts of phosphorus removed from the soil were as follows:

Primary root of plant I	: 163029cpm (44242 cpm from within the root hair cylinder)
Primary root of plant II	: 104280 cpm (23288 cpm from within the root hair cylinder)
Laterals	: 32747 cpm
Total	: 300056 cpm

The extent of agreement between the amount removed from the soil and that found within the plants (369474 cpm in the aerial parts) is satisfactory, considering (i) the approximation introduced by measuring concentrations at points 0.05 cm apart and (ii) the impossibility of measuring the small contributions, if any, from the laterals which grew into the soil block.

Phosphate gradients around roots

An example of the phosphate gradients is shown in Fig. 4. The autoradiograph scan across root II at 0.7 cm from the soil surface is superimposed on the theoretical gradients calculated for (i) the

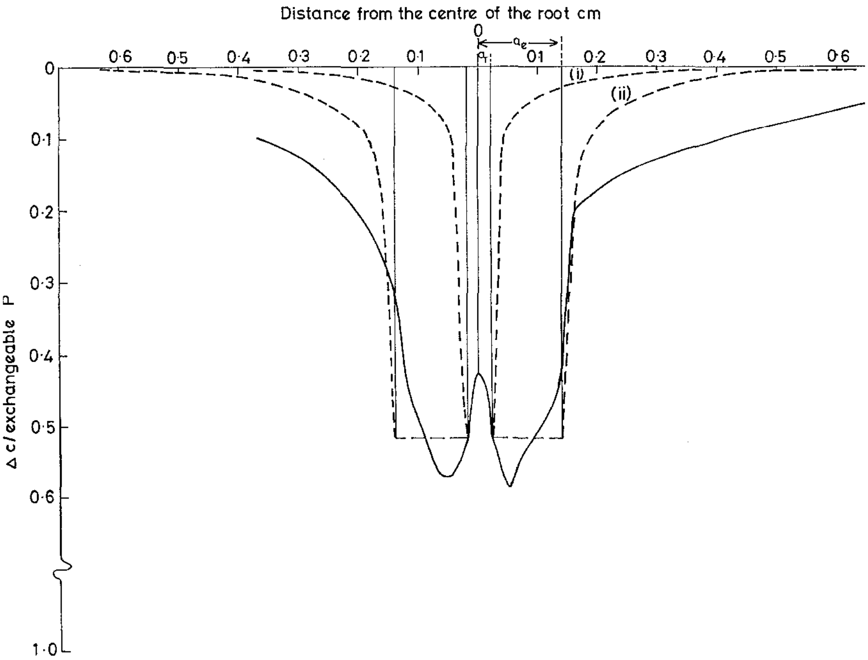

Fig. 4. Exchangeable P gradient around primary root II at 0.7 cm from soil surface:

broken line (*i*): calculated assuming the root hairs as inactive
broken line (ii): calculated assuming intense root hair activity and uniform depletion from within the root hair cylinder
solid line: observed in the experiment.

primary root axis, assuming the root hairs are inactive, and (ii) the root hair cylinder, assuming intense root hair activity and uniform concentration within the root hair cylinder. These theoretical gradients were computed numerically, using a modification of the program of Nye and Marriott [10] that allowed for the variation of C with C_l according to equation (1). The value of the root absorbing power was adjusted to give the experimental value of ΔC at the surface of the root axis or the root hair cylinder so that the program only predicts the spread of the depletion curve. The value of the root absorbing power required was 10^{-1} cm sec^{-1}.

It is clear from this figure that the experimentally observed gradient is much closer to (ii) than to (i), a very sharp drop in the concentration of exchangeable P being observed at a point near the surface of the root hair cylinder. This was observed at other points of scanning as well, except at less than 3-day old parts of the root, where there was a continuous and gradual drop in concentration within the root hair cylinder itself, showing that in those regions, the root hairs were continuing to grow in length and in number when the experiment was terminated.

On this evidence uptake from within and without the root hair cylinder will be discussed separately.

Uptake from within the root hair cylinder

The amounts of P depleted from within the cylinders of the two primary roots are presented in Fig. 5(a). The same results expressed as fractions of the total amounts of exchangeable P initially contained in the same soil volume are given in Fig. 5(b). The absorptions from about 1.5 cm length of root from the tip was negligible.

These results show a rapid depletion from within the root hair cylinder within a short absorption period, after which, the change in concentration is only slight. The total amounts absorbed then depend on the dimensions of the root hair cylinder, as is clear from the comparability of Figs. 5(a) and 3(b). It appears that only about 60 per cent of the P exchangeable in 7 days is taken up by the plant. The corresponding concentrations of phosphorus in solution, obtained from the isotherm were extremely low at root lengths more than 2 days old.

It may be argued here that the seven day period of equilibration with P^{33} is inadequate. The period is in fact a compromise. A long period of equilibration would have resulted in depletion of a correspondingly smaller fraction of exchangeable P, and consequent loss of accuracy. Although isotopic equilibrium has not been achieved during the 7 day period, the conclusions of this experiment will not be affected to any considerable extent as long as the rate of depletion of P greatly exceeds that of any isotopic exchange with the less readily exchangeable fractions of soil P which might have continued during the period of plant growth. That this has been so in this experiment is evident from the constancy of the fraction of exchangeable P taken up by the plant from within the root hair cylinder at regions more than 2 days old, especially for root I. Any significant isotopic exchange of the slowly exchangeable soil P would be accompanied by a corresponding decrease in the fractions of exchangeable

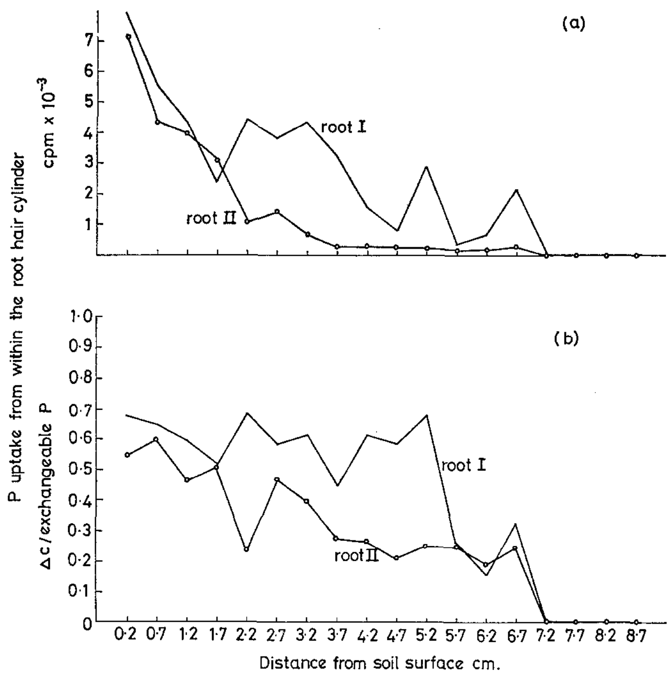

Fig. 5. P uptake from within the root hair cylinders of the two primary roots at the points of scanning expressed as: (**a**) total amounts, (**b**) fractions of total exchangeable P initially contained in the soil volume within the cylinder.

P taken by the plant at the younger regions of the root hair cylinder, for a given period of absorption.

Uptake from outside the root hair cylinder

The rapid initial depletion from within the root hair cylinder and the relative constancy of the P concentration within it thereafter, offer the possibility of predicting the amount of the nutrient moving into it from outside – considering the root hair cylinder in this experiment as a hollow semicylinder with a uniform surface concentration. The theoretical uptake from outside the cylinder was calculated for the entire root length using the formula (see Nye[9], p. 100)

$$\tfrac{1}{2}\pi a_e^2 l \Delta C \left[\frac{4}{\sqrt{\pi}} \left((D)t/a_e^2\right)^{\frac{1}{2}} + (D)t/a_e^2 \right] \qquad (3)$$

where

a_e is the radius of the equivalent root hair cylinder in cm
l the length of root section considered in cm
ΔC the decrease in concentration of P within the cylinder in cpm ml^{-1} soil
(D) the effective diffusion coefficient as obtained from Equation (2) in $cm^2\ sec^{-1}$
and
t the age of the root section in sec

The predicted as well as the experimentally determined uptakes of P from outside the root hair cylinder are given in Fig. 6. The predicted and observed total amounts were 50666 and 118787 cpm for plant I and 36607 and 80992 cpm for plant II respectively.

In predicting the amounts of P diffusing into the root hair cylinder from outside, all the parameters in Equation (3), except the effective diffusion coefficient (D), are measured with satisfactory accuracy. The accuracy of (D) which varied between 0.7×10^{-8} and $2.5 \times 10^{-8}\ cm^2\ sec^{-1}$ in this experiment depends on the values of $\Delta C_l/\Delta C$ in Equation (2), which are derived from the desorption isotherm. Although the conditions of labelling the exchangeable P and the operating temperature were identical with those in the ex-

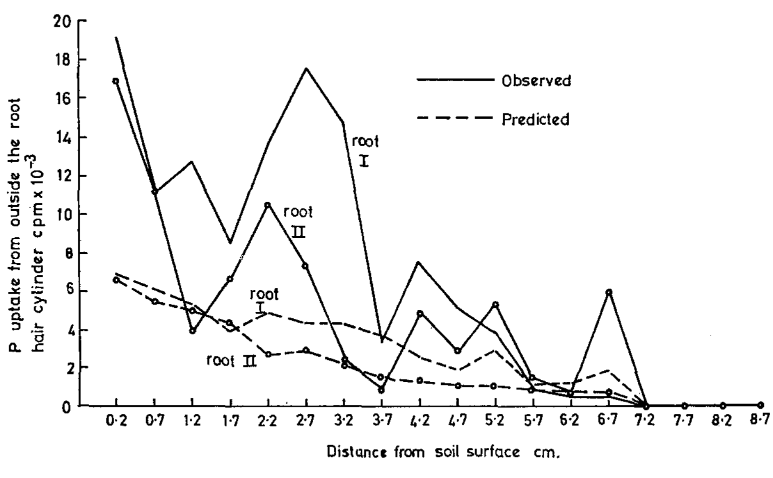

Fig. 6. Predicted and observed uptakes of P from outside the root hair cylinders of the two primary roots at varying depths from the surface of the soil block.

perimental soil block, the equilibrium between the soil and the solution might still be different in undisturbed soil from that obtained by shaking the same soil with large volumes of solution for 24 hours. This is especially so in the root region because of possible continuous changes in $\Delta C_l/\Delta C$ due to rhizosphere effects. There were no indications of an increased microbial activity at the root surface [2], such as localised zones of higher activity on the scans. Further, since the major part of the P removed from the soil was found in the aerial parts of the plants, microbial interference if any, does not seem to be important in this experiment. However, changes in pH in the rhizosphere caused by root secretions, or by a differential uptake of anions and cations by the plant cannot be ruled out. Significant effects of pH on the diffusion coefficients of P have been reported by Place *et al.*[12] in all soils and clays they studied, under conditions of low microbial activity. Unpublished results of experiments on the soil used in the present study as well as on others [1], have shown considerable changes in the slopes as well as origins of the isotherms with the operating pH. The need for a better understanding of the modifications of the isotherm by the various factors operating in the rhizosphere over periods of uptake of several days is thus obvious.

Unlike the present calculations, predictions of uptake that are based directly on solution concentrations are not very sensitive to changes in $\Delta C_l/\Delta C$ in cylindrical systems (see Nye[9], p. 91). Hence, the consistent results reported in earlier studies in which predictions were made from such calculations [5 11] do not in themselves adequately evidence that the correct values for $\Delta C_l/\Delta C$ were used, especially since the concentration at the root surface was estimated from experimentally observed uptakes. As can be seen from Equation (3) the theoretical uptakes calculated here are, on the contrary, highly sensitive to (D) which in turn directly depends on $\Delta C_l/\Delta C$.

Based on the agreement, involving considerable extrapolation, between the phosphate diffusion coefficients measured by a method that makes use of a capacity factor and one that does not, Olsen and Watanabe [11] claim to have used a correct value for the parameter (B), which relates the labile P to the solution P in their predictions. Their experiments were conducted with 6 cm lengths of preformed roots and the maximum period of absorption was 24 hours. Over longer periods, and with actively growing roots, changes in the rhizosphere are likely to have much greater consequences on the parameter (B).

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