to 8.5. At pH values of 3 and 10.5 there were marked reductions in the rate of respiration. Roots left 16 hours in a nutrient solution buffered at pH 9.4 showed only a slight decrease in rate of oxygen uptake.

The respiration of excised bean roots immersed in Hoagland's solution was not affected appreciably by Cl^-, $SO_4^=$, Ca++, and Na+ ions at concentrations of 25 meq/l. Bicarbonate ions inhibited the respiration markedly. The inhibition increased with time.

The rate of oxygen consumption by excised roots from a group of plants susceptible to lime-induced chlorosis was markedly reduced by bicarbonate ions. Roots from plants resistant to lime-induced chlorosis were only slightly affected by bicarbonate.

The inhibition of oxygen uptake by carbon monoxide with roots in standard Hoagland's solution was largely reversed by light, but in the presence of bicarbonate ions the carbon monoxide inhibition was only partially reversed by light.

Bicarbonate inhibition of respiration of excised roots is not fully explained by a direct inhibition of the cytochrome system.

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ABSORPTION OF IONIC SPECIES OF ORTHOPHOSPHATE BY BARLEY ROOTS: EFFECTS OF 2,4-DINITROPHENOL AND OXYGEN TENSION[1,2]

H. T. HOPKINS

Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

The linkage between active salt absorption by plant roots and oxygen tension has been described by Hoagland and Broyer (6) and Steward et al (15). Despite these contributions, more detailed experiments employing shorter absorption periods and oxygen partial pressures below one percent are desirable. Information is needed with respect to orthophosphate, in particular, since early workers studied the absorption of bromide, nitrate, and potassium ions, as influenced by anaerobiosis.

From experiments described in this paper, it is found that each of the two first order reactions through which $H_2PO_4^-$ and $HPO_4^=$ ion species are absorbed (5), have an oxygen requirement. The overall rate of phosphate sorption is shown to be independent of the partial pressure of oxygen over the range from about 3 to 100 % oxygen, where the total pressure is one atmosphere. Another finding is that each of the two first order reactions is inhibited competitively by 2,4-dinitrophenol.

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Materials and Methods

Seed of barley, *Hordeum vulgare,* var. Atlas 46, were obtained from the 1954 crop grown in the vicinity of Davis, California. Twenty grams of seed were soaked for 24 hours in approximately one liter of demineralized water, aerated at a flow-rate of 1000 ml per hour at 24° C in a dark chamber. The sprouted seeds were then rinsed in demineralized water and distributed on boiled cheesecloth supported on a stainless steel screen and covered as described previously (5). Seedling roots were grown five days at 24° C in darkness in 1×10^{-4} M $CaSO_4$ solutions, aerated at a flow-rate of 2500 ml per hour per liter of solution, using glass tubing aerators with an inside diameter of 3 mm. Healthy roots 10 to 15 cm in length were obtained with this procedure. Roots were excised with clean

shears about 5 mm below the seeds. Excised roots were rinsed and placed in about 3 liters of demineralized water and thoroughly mixed. Experiments were performed using one gram of roots in phosphate solutions in equilibrium with nitrogen : oxygen gas mixtures containing oxygen at partial pressures ranging from 0.002 to 21 % oxygen.[3] Argon gas was not a contaminant in any of these mixtures.

Most of the dissolved oxygen was removed initially from demineralized water by boiling; then the gas mixtures were bubbled through either one liter volumes contained in Erlenmeyer flasks having standard taper joints, or 70-ml test tubes also fitted with standard taper joints. The gases escaped from the containers via exit tubes under the water surface in the water baths. On the basis of information received from workers at the Bureau of Standards, it was learned that where the flow rate was 2500 ml per hour, a one liter volume would reach equilibrium with the influent gas after 15 hours. Where the flow-rate was four-fold higher, a one liter volume would equilibrate after 3 hours, provided stoppers were employed as well as other precautions. In most cases, a flow-rate of 2500 ml per hour was found most convenient for use.

On leaving the steel cylinders, the gas mixtures passed through a scrubbing tower containing water, then through a cotton filter and into a copper or glass manifold. From this point the gas mixture flowed into the phosphate solutions. No more than three thick walled rubber tubing connectors were used in any gas line assembly. All joints in the copper manifold were carefully soldered, and no leaks could be detected in the assembly under the positive pressure head of about one pound used in these experiments.

Absorption experiments were usually done using one liter volumes in order to maintain the phosphate concentration and the pH essentially unchanged during one hour absorption periods. However, experiments in which solutions containing phosphate at 1×10^{-4} M and pH 4 were invariably done in 50 ml volumes, since changes in concentration were negligible and pH control was easily maintained during one-hour periods. In experiments with 2,4-dinitrophenol, experimental design was similar. DNP, obtained from Eastman Kodak Company, was made up in two stock solutions containing 18.4 and 184 μg DNP per ml respectively; from these, solutions were made ranging from 1×10^{-7} M to 1×10^{-3} M.

In preliminary experiments a system was employed where the phosphate solutions and the roots were emptied independently into the vessels following a period of time during which the entire assembly came to equilibrium with any particular gas mixture. The assembly was fitted with standard taper joints and adapters such that side-arm flasks could be swung independently through an arc of 180 degrees to permit emptying the contents of the side arm flasks into the main vessel of one liter volume. Through the use of this set-up, it was found that prior equilibration of roots with the liquid phase in equilibrium with low oxygen tensions was not critical. The critical point was the preparation and maintenance of solutions essentially in equilibrium with a given gas mixture. Choice of flow-rates was important in this respect.

The pH of solutions was adjusted to the desired value with 0.1 *N* KOH or HCl. The one-liter Erlenmeyer flasks or 70-ml test tubes containing the various solutions were placed in a water bath at 30° C and the solutions, under constant agitation of the inflowing gas mixture, came to the temperature of the water bath. Under aerobic conditions, the rate of phosphate absorption remained constant over the flow-rate range from 2,000 to 30,000 ml per liter × hour.

In all experiments the specific activity of the solutions used in absorption studies was 3.2 mc per gram P. A radioactive phosphate stock solution was made up beforehand to contain 40,000 mc per gram P, as described previously (5). At a given oxygen pressure or DNP concentration, phosphate was varied over the range from 10^{-4} M to 10^{-6} M, with the pH adjusted to 4 or 7.

Excised roots were removed from demineralized water in which they had been suspended following excision, blotted on cheesecloth, and one-gram portions weighed out on a torsion balance. At zero time, the roots were transferred to solutions containing the desired amount of tracer phosphate, provided sufficient time had elapsed for full equilibration of the liquid phase with the gas mixture. In all experiments, the maximum length of the absorption period was 60 minutes, unless otherwise indicated. At the end of absorption periods, the roots were rinsed and then transferred to solutions containing inactive phosphate and exchanged for 60 minutes, following which the roots were rinsed and dried under an infra-red lamp. Activity in the dry root samples was measured in a proportional counter using 90 % argon and 10 % methane gas purge. From preliminary experiments it was found that phosphate absorbed under anoxic conditions was bound in essentially an irreversible manner.

Design of Experiments: Ion absorption has been shown to be consistent with the following generalized scheme (3),

(*1*) The ion (M) combines with a metabolically produced carrier (R) to form a reversibly dissociable complex (MR); i.e., $M + R \rightleftharpoons MR$.

(*2*) The intermediate complex (MR) then breaks down, resulting in the liberation of the ion (M), and the regeneration of the carrier (R):

$$MR \rightleftharpoons M_{inside} + R_{regenerated}$$

From the expressions in (*1*) and (*2*), an equation can be derived which is formally analogous to that derived for enzyme kinetics by Michaelis and Menten (12). Lineweaver and Burk (9) later showed that on plotting the reciprocal, 1/v on the ordinate against the reciprocal 1/S on the abscissa, the results fall on a straight line as expressed by the linear function in equation (*3*).

[3] The amount of oxygen in each gas mixture was verified by the Gas Chemistry Section, Bureau of Standards.

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}} \quad (3)$$

where (v) denotes the moles phosphate absorbed in one hour by one gram of excised barley roots; (S) denotes total phosphate concentration, hereafter expressed [ΣP]; V_{max} is the maximum absorption at infinite phosphate concentration, and K_m corresponds to the equilibria between phosphate and its carrier, or the apparent dissociation constant of the carrier-phosphate complex, under conditions where interfering ions are absent (5). In recent work, it was found that phosphate absorption could not be interpreted as consisting of a single first order reaction (5). These findings are confirmed in this work.

A method was developed by Hofstee (7), for determining whether more than one independent reaction is acting on a single substrate, or on different substrates which are in a non-rate limiting equilibrium. This method has been applied in the present work. By plotting the observed absorption v, against v/[ΣP], one obtains a curving line like that for the control line

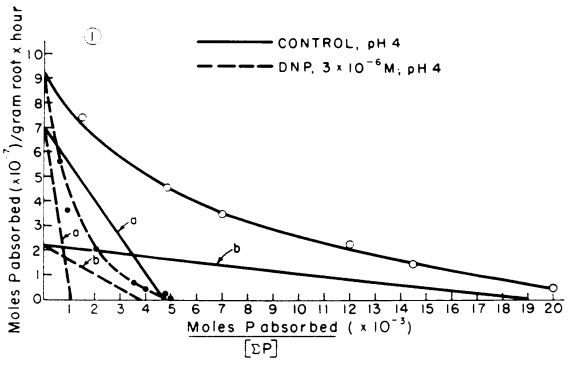

FIG. 1. Graphical separation of reactions involved in phosphate absorption at pH 4, in presence and absence of 2,4-dinitrophenol.

in figure 1. The curve may be resolved into linear components by use of polar coordinates, but in the case where the dissociation constants, K_m, differ widely from one another, the function can be solved by extrapolation as described previously (5). Intercepts with the ordinate for the two first order reactions, a and b, give V_{max} directly, and the values of the slopes give apparent dissociation constants, K_m, in respect to total phosphate concentration, [ΣP]. Since K_m expresses the equilibria between specific ion species and respective carriers, the constants have to be calculated in terms of the concentration of each ion species, $H_2PO_4^-$ and $HPO_4^=$. Knowing already the relative concentration of each ion species at a given pH value, one recalculates the slope of each line, a and b in figure 1, on the basis of the total concentration of each ion species, by use of the method of least squares (14). In order to determine the manner in which molecular oxygen and the inhibitor 2,4-dinitrophenol enter phosphate absorption reactions, it is necessary to extend the kinetic analysis discussed above. A critical discussion of three types of inhibitions can be found in the paper by Epstein and Hagen (3). Other types of inhibitions are known and have been described (4). A convenient method for distinguishing competitive from non-competitive inhibition is afforded by determining if the slope of the line increases in the reciprocal plot 1/v versus $1/[H_2PO_4^-]$, or $1/[HPO_4^=]$, while the intercept remains constant, as for example in figure 2. In non-competitive inhibition, the ratio between reciprocal slope and intercept remains essentially constant, while in competitive inhibition, the ratio increases, tables I and II, columns 2 and 3.

EXPERIMENTAL RESULTS

An important feature of experiments dealing with agents which can alter reaction rates is the unequivocal demonstration of reversibility in the system under study. With respect to phosphate absorption by barley roots, it can be shown that the inhibited reaction can return to an absorption rate typical of uninhibited roots. Results in figures 3 and 4 were obtained from experiments employing a range of concentrations of molecular oxygen, or 2,4-dinitrophenol. Roots were placed in solutions in equilibrium with various oxygen partial pressures, with phosphate at 1×10^{-4} M, pH 4, for intervals of 60 to 75 minutes, curves a and b, figure 3. Following these time intervals, air was introduced into the solutions and absorption went on under aerobic conditions for an additional 90 minutes. Results from similar experiments, employing oxygen tensions of 0.3 % and 21 % oxygen are also shown in figure 3, curves c and d. Apparently, the absorption mechanism is altered in an irreversible manner following 75 minutes exposure in solutions in equilibrium with an oxygen tension of 0.05 %, curve a. Following a 60-minute exposure to 0.05 % and 0.3 % oxygen, curves b and c, a steady rate is attained equal to that for fully aerobic roots, curve d. The initial absorption "lag" observed in the first leg of curves a, b, and c, was found to occur equally at pH 7 as at pH 4. The possibility that the "lag" was due to a slow rate of saturation of the external solution with air, or to excretions of unknown compounds from anoxic roots, was ruled out by replacing solutions present at the end of the anaerobic periods with fresh solutions in equilibrium with air. No change occurred in the character of the aerobic recovery curves over a 90-minute period.

Results in figure 4 were obtained with varying DNP concentrations. Following a 60-minute absorption period in "aerobic" solutions containing known amounts of DNP, and phosphate at 1×10^{-4} M, the roots were rinsed and placed in "aerobic" solutions containing phosphate 1×10^{-4} M, pH 4, for 90 minutes. The absorption reactions are reversible following DNP treatments at and below concentrations of 3×10^{-6} M, curves c and d, figure 4. The absorption rate following 60 minutes in DNP, 1×10^{-5} M, pH 4, was one-third the normal rate, curves a and e. Phosphate absorption is approximately half-maximal at a DNP concentration of 3×10^{-6} M in solutions containing phosphate 1×10^{-4} M, pH 4.

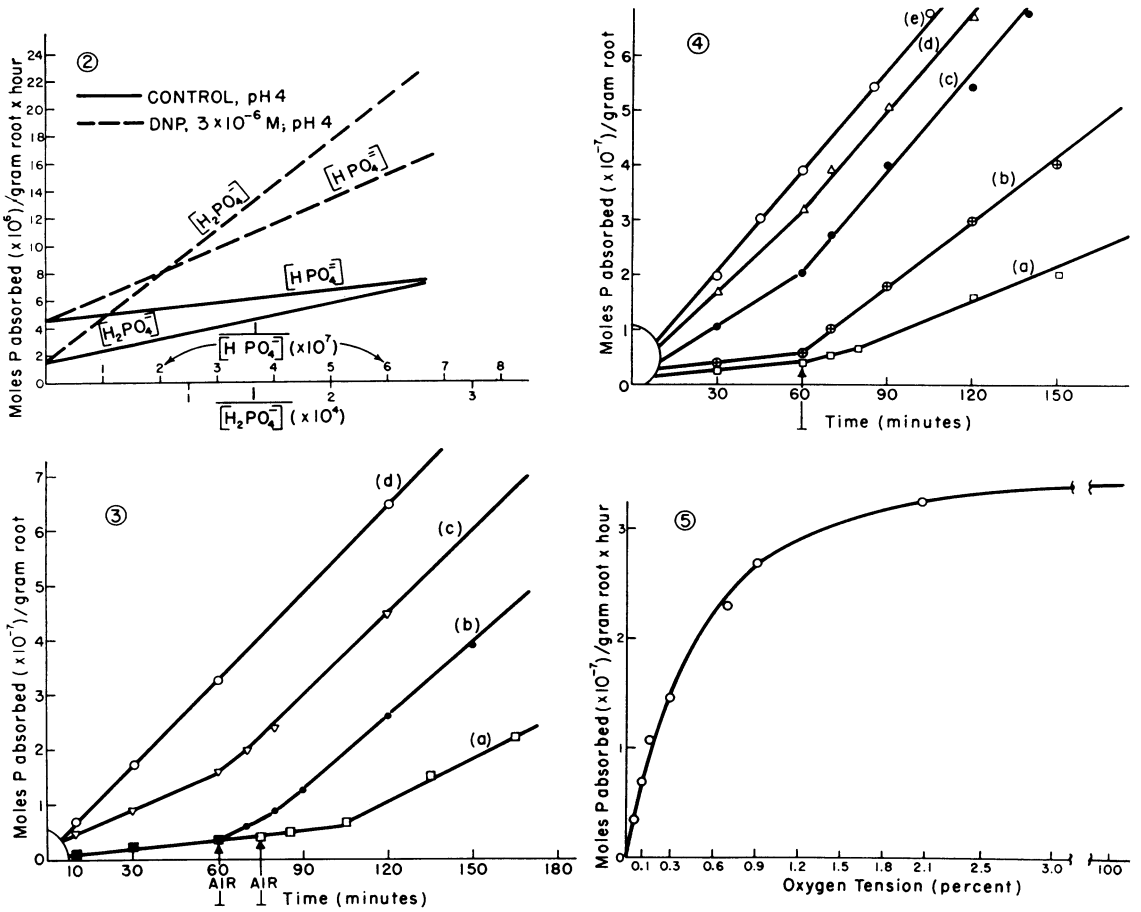

FIG. 2. Calculated curves showing competitive inhibition of the absorption of ionic species of orthophosphate by DNP 3×10^{-6} M, pH 4.

FIG. 3. Reversibility of phosphate absorption reactions following anoxic exposure; in curve a, arrow indicates introduction of air after 75 min exposure to 0.05 % oxygen; in curves b and c, air introduced after 60 min exposure to 0.05 % and 0.3 % oxygen, respectively; curve d, phosphate absorption in air. In each case, phosphate concentration was 1×10^{-4} M, and pH 4.

FIG. 4. Reversibility of phosphate absorption reactions following 60 min exposure to various concentrations of 2,4-dinitrophenol. Arrow indicates point at which roots were rinsed and returned to phosphate solutions 1×10^{-4} M, pH 4. Curve a, DNP 1×10^{-5} M; curve b, DNP 5×10^{-6} M; curve c, DNP 3×10^{-6} M; curve d, DNP 2×10^{-6} M; curve e, control.

FIG. 5. Relation between oxygen tension and phosphate absorption by excised barley roots in phosphate solutions 1×10^{-4} M, pH 4.

Between the limits from 5 to 60 minutes, absorption rate is linear and reversible at all oxygen tensions from 0.05 % and above, and all DNP concentrations at and below 3×10^{-6} M. Barley roots used in these experiments become flaccid after 25 minutes exposure to 0.002 % oxygen in solutions containing phosphate 1×10^{-4} M, pH 4. Similarly, 2 to 3 hours exposure to 0.05 % oxygen or DNP concentrations greater than 5×10^{-6} M causes the roots to become flaccid and defunct (unpublished data).

Absorption rate is independent of oxygen partial pressure over the range from about 3 to 100 % oxygen, figure 5. A hyperbolic relationship exists between the phosphate absorption rate and oxygen tension between the limits of 0 and 3 %. At an oxygen tension of 0.30 %, phosphate sorption rate is half-maximal, and maximal at 3.0 %, in solutions 1×10^{-4} M with respect to phosphate and at pH 4.

FURTHER EXPERIMENTS WITH 2,4-DINITROPHENOL: The effect of DNP on phosphate absorption was studied using phosphate concentrations over the range from 10^{-6} M to 10^{-4} M, at pH 4 or pH 7, and at one or more DNP concentrations. When the observed absorption during one hour, v, is plotted against the variable $v/[\Sigma P]$, one obtains the curve shown in figure 1. The curve may be resolved into its linear components as described previously under Methods. Intercepts with the ordinate for the two first order reactions a and b, give V_{max} directly for each reaction, and the values of the slopes give ap-

parent dissociation constants, K_m, in respect to total phosphate concentration [ΣP]. As previously explained, when one recalculates the slope of each line on the basis of the total concentration of the specific ion species, it is possible to re-plot the function in terms of a conventional Lineweaver and Burk plot, as shown in figure 2. Inspection of these reciprocal plots shows that DNP competitively inhibits the absorption of both $H_2PO_4^-$ and $HPO_4^=$ ions, thus indicating that DNP is bound at the same reactive group on the carrier as the phosphate ion species.

Quantitative expression is given to the manner in which DNP enters phosphate absorption reactions in table I, columns 2, 3, 6, and 7, in terms of numerical values for reciprocal intercept and slope. The apparent dissociation constants corresponding to the equilibria between $[H_2PO_4^-]$ and $[HPO_4^=]$ and their respective carriers $[R_a]^+$ and $[R_b]^+$ for systems without the inhibitor are given in columns 4 and 7. Similar values corresponding to the equilibria for systems containing DNP are given in columns 5 and 9. In the presence of DNP, 3×10^{-6} M, pH 4, the slope increases and the intercept remains unchanged from that of the untreated system. Results in figure 2 show this in a graphic manner, and indicate further that the inhibitory effect of DNP is abolished by increasing concentrations of the specific ion species. Apparently, the affinity of DNP for each of the intermediate complexes is of the same order of magnitude, table I, columns 5 and 9. The concentration of DNP producing half-maximal inhibition is 3×10^{-6} M, in solutions 1×10^{-4} M with respect to phosphate, pH 4. At pH 7, half-maximal inhibition occurs at a DNP concentration of 1×10^{-4} M, representing a thirty-fold higher concentration of DNP (unpublished data).

FURTHER EXPERIMENTS WITH MOLECULAR OXYGEN: In this study, molecular oxygen is considered an inhibitor, though in a sense which is the reverse of that usually applied. For instance, phosphate sorption by barley roots is a hyperbolic function of the

TABLE I

COMPETITIVE INHIBITION OF ABSORPTION OF IONIC SPECIES OF ORTHOPHOSPHATE BY 2,4-DINITROPHENOL, pH 4

	[2,4-DINITROPHENOL]	
	NONE	3×10^{-6} M
	($R_aH_2PO_4$)	
Intercept, $\frac{\text{Moles}^{-1}}{\text{M/L}^{-1}}$ ($\times 10^6$)	1.42	1.48
Slope, Moles^{-1} ($\times 10^2$)	2.07	7.86
K_m, Moles ($\times 10^{-4}$)	1.46	...
K_i, Moles ($\times 10^{-6}$)	...	1.14
	(R_bHPO_4)	
Intercept, $\frac{\text{Moles}^{-1}}{\text{M/L}^{-1}}$ ($\times 10^6$)	4.50	4.50
Slope, Moles^{-1} ($\times 10^{-2}$)	3.80	16.63
K_m, Moles ($\times 10^{-9}$)	8.44	...
K_i, Moles ($\times 10^{-6}$)	...	1.01

TABLE II

"NON-COMPETITIVE" NATURE OF THE OXYGEN EFFECT ON ABSORPTION OF IONIC SPECIES OF ORTHOPHOSPHATE, pH 4

	OXYGEN PARTIAL PRESSURE		
	21 %	0.71 %	0.30 %
	($R_aH_2PO_4$)		
Intercept, $\frac{\text{Moles}^{-1}}{\text{M/L}^{-1}}$ ($\times 10^6$) ..	2.08	3.53	5.44
Slope, Moles^{-1} ($\times 10^2$)	1.25	2.27	2.78
K_m, Moles ($\times 10^{-5}$)	6.00	...	...
K_i, Moles ($\times 10^{-5}$)	...	2.60	1.20
	(R_bHPO_4)		
Intercept, $\frac{\text{Moles}^{-1}}{\text{M/L}^{-1}}$ ($\times 10^6$) ..	19.60	27.40	28.90
Slope, Moles^{-1} ($\times 10^{-2}$)	3.90	7.20	8.00
K_m, Moles ($\times 10^{-9}$)	2.00	...	...
K_i, Moles ($\times 10^{-5}$)	...	6.50	3.80

degree of saturation of an unspecified site with molecular oxygen, between the limits of oxygen partial pressures from about 3 to 0 %, figure 5. As in the experiments with DNP, quantitative expression is given to the manner in which molecular oxygen enters phosphate absorption reactions, by the extent of change in the reciprocal intercept and slope when oxygen becomes limiting, table II. Both intercept and slope change each time oxygen tension changes, and by approximately the same amount as shown in columns 2, 3, 6, and 7. In a simple system, this would be analogous to a non-competitive inhibition and would indicate that molecular oxygen combines with both the carrier and the carrier-phosphate intermediate complex at a site or reactive group different from that occupied by phosphate ions. This behavior implies that the response of the phosphate absorption reactions to oxygen stress is independent of the phosphate concentration in the external medium. This was indeed found to be the case over the range of phosphate concentrations from 2×10^{-6} to 4×10^{-4} M, at pH 4 or 7. Caution should be exercised, however, in broadly generalizing from application of kinetic analysis, developed originally for simple systems, to complex systems like the phosphate absorption mechanism. At the cellular level, for instance, one is usually working with specific binding sites separated spatially within a reaction sequence.

Results shown in table II, columns 5 and 9, indicate that the affinity of molecular oxygen is of the same order of magnitude for each of the carriers $[R_a]^+$ and $[R_b]^+$. The maximum absorption of the ionic species of orthophosphate at infinite phosphate concentration, V_{max}, is shown in table III. Absorption of $H_2PO_4^-$ is lowered to a slightly greater extent than $HPO_4^=$ at all oxygen tensions below 21 %.

DISCUSSION

Certain assumptions shown to be valid from previous work are applied implicitly in this study (5).

TABLE III

MAXIMUM ABSORPTION OF IONIC SPECIES OF ORTHOPHOSPHATE, V_{max}, IN RELATION TO OXYGEN TENSION, pH 4

OXYGEN PRESSURE, %	$V_{max}[R_aH_2PO_4]$ Moles ($\times 10^{-8}$)	PERCENT OF CONTROL	$V_{max}[R_bHPO_4]$ Moles ($\times 10^{-8}$)	PERCENT OF CONTROL
21.0	48.1×10^{-8}	100	5.1×10^{-8}	100
0.71	28.3×10^{-8}	59	3.6×10^{-8}	71
0.30	18.4×10^{-8}	38	3.5×10^{-8}	69
0.05	6.0×10^{-8}	12	1.3×10^{-8}	25

Briefly, these assumptions are that, 1) one mole of phosphate ion species may combine with the carrier; 2) the rate of phosphate absorption is proportional to the concentration of the carrier-phosphate complex; the uptake rate reaching a maximum value when the carrier is saturated with phosphate; and 3) the rate-limiting step is the breakdown of the intermediate complex.

In this study, the kinetic analysis is made under conditions where the results from inhibitor experiments accurately measure the rate-limiting step. Furthermore, it is desirable to point out that barley roots are favorable material for use in studies relating oxygen tension to a process such as phosphate sorption. In the first place, the radius of the barley roots used in this work is approximately 0.02 cm. Calculations reveal that the rate of oxygen diffusion into root cells cannot be a limiting factor in these experiments.

2,4-Dinitrophenol competitively inhibits the absorption of both $H_2PO_4^-$ and $HPO_4^=$ ions under conditions where hydroxyl ions do not compete, namely; pH 4. This finding may offer a clue to the isolation of the carrier-phosphate complexes in barley roots. Dinitrophenol has long been known to produce an uncoupling of phosphorylations from respiration (10). Speculation concerning the significance of the competitive inhibition of phosphate absorption by DNP is unprofitable, since the site at which DNP acts has not been determined (1). Furthermore, the relationship between oxidative phosphorylations via the respiratory chain, and phosphate sorption by barley roots remains to be established.

Results from experiments where pH is varied between 4 and 7, show that the penetration of membranes by DNP is facilitated at pH 4. This finding is in agreement with the work of Beevers (2), and Newcomb (13), both of whom studied the effect of DNP on respiration. Presumably, penetration occurs as the undissociated molecule, following which DNP ionizes and combines with each of the two carriers with equal affinity.

At 30° C phosphate absorption is half-maximal at an oxygen partial pressure of about 0.3 %, figure 5. This value for phosphate absorption in relation to oxygen tension is much lower than that previously reported for bromide and potassium ions (6). According to Ludwig and Kuby (11), half-maximal oxidation of cytochromes a_3, a, and c, occurs in yeast cells at an oxygen tension of about 0.2 % at a temperature of 25° C. This correlation is submitted as suggestive evidence that cytochrome oxidase is the terminal oxidase in the six-day-old barley roots used in this study. Temperature has a profound influence on the affinity between cytochrome oxidase and molecular oxygen (8).

Information on the equivalence, in moles, between molecular oxygen and the carriers $[R_a]^+$ and $[R_b]^+$ is obtained by application of equation (4) to the results of figure 5.

$$\frac{v}{v_i} = 1 - \frac{(I)^r}{K_i}, \quad (16), \tag{4}$$

where (v) is absorption rate in air, (v_i) is absorption rate at some oxygen tension lower than 3 %; (I) is the oxygen tension, and (r) is the number of molecules of oxygen combining with one molecule of the carrier. The value for (r) is determined by estimating the slope of the line obtained when log $((v/v_i) - 1)$ is plotted against log (I). In the overall reaction, it is found that 1.1 moles of oxygen combine with both carriers. When the overall reaction is broken down into each of the two first order reactions, one finds that approximately 20 % less oxygen is required for the reaction involving $HPO_4^=$ than that for the $H_2PO_4^-$ ion.

SUMMARY

The effect of oxygen tension and 2,4-dinitrophenol on phosphate absorption by excised roots of *Hordeum vulgare*, variety Atlas 46, was studied. Quantitative expression is given to the manner in which molecular oxygen and dinitrophenol enter phosphate absorption reactions.

1. Dinitrophenol competitively inhibits the absorption of both $H_2PO_4^-$ and $HPO_4^=$ ions.

2. Phosphate sorption is independent of oxygen tension over the range from about 3 to 100 %, where the total pressure is one atmosphere.

3. At an oxygen tension of 0.3 %, phosphate absorption rate is half-maximal, and over the range from 3 to 0 % oxygen tension, the relation is typically a hyperbolic function.

4. At an oxygen tension of 0.71 %, the maximum rate of phosphate absorption at infinite phosphate concentration for the first order reaction involving $[H_2PO_4^-]$ was lowered 41 % and that for $[HPO_4^=]$ was lowered 29 %. Hence, both first order reactions have an oxygen requirement.

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