

# Relationship between Energy-dependent Phosphate Uptake and the Electrical Membrane Potential in *Lemna gibba* G1<sup>1, 2</sup>

Received for publication May 5, 1980 and in revised form July 9, 1980

CORNELIA I. ULLRICH-EBERIUS, ANTON NOVACKY<sup>3</sup>, ELKE FISCHER<sup>3</sup>, AND ULRICH LÜTTGE  
*Institut für Botanik der Technischen Hochschule Darmstadt, Schnittpahnstrasse 3 - 5, D-6100 Darmstadt,  
Federal Republic of Germany*

## ABSTRACT

High rates of phosphate uptake into phosphate-starved *Lemna gibba* L. G1 were correlated with a high membrane potential ( $pd = -220$  millivolts). In plants maintaining a low  $pd$  ( $-110$  millivolts), the uptake rate was only 20% of that of high- $pd$  plants. At the onset of phosphate transport, the membrane of high- $pd$  plants was transiently depolarized. This effect was much smaller in low- $pd$  plants. Light stimulated phosphate uptake and the repolarization upon phosphate-induced depolarization, especially in plants grown without sucrose. The phosphate uptake rate was optimal at pH 6 and decreased with increasing pH, corresponding to the phosphate-induced  $pd$  changes. Phosphate starvation stimulated the uptake and increased the phosphate-induced depolarization, thus indicating that phosphate uptake depends on the intracellular phosphate level. It is suggested that uptake of monovalent phosphate in *Lemna gibba* proceeds by an  $H^+$  cotransport dependent on the proton electrochemical potential difference and, hence, on the activity of an  $H^+$ -extrusion pump.

Phosphate uptake by unicellular algae is stimulated up to 10-fold by  $Na^+$  or  $Li^+$  ions in the neutral and alkaline pH range (23, 24). The same was found in fungi (18, 19) and was interpreted as a  $Na^+$ -phosphate co-transport system (18). In addition, it has been shown in yeast and in bacteria that, in the acidic pH range, phosphate absorption is accompanied by an  $H^+$  influx with a stoichiometry of 1.5 to 3  $H^+$ /phosphate transported (2, 5, 8). Phosphate uptake seems to proceed by a cotransport mechanism along an electrochemical transmembrane  $Na^+$  or  $H^+$  potential gradient similar to the described sugar- $H^+$  cotransport in lower plants (10, 20).

For higher plants, it was suggested that an  $H^+$  cotransport occurs with sugars, amino acids, and nitrate (15, 22). This was concluded from transient alkalization of the external medium and from the transient depolarization of the plasmalemma at the onset of solute transport (7, 14-16, 22). The results were regarded to be consistent with the hypothesis of Mitchell (13), who proposed a solute- $H^+$  cotransport along  $\Delta\mu H^+$  through bacterial mem-

branes, driven by an  $H^+$ -extrusion pump.

In higher plants, phosphate uptake was highly reduced in an alkaline medium and not stimulated by  $Na^+$  (25). In the experiments presented here, we investigated if energy-dependent phosphate uptake in *Lemna*, an autotrophic higher plant, might be maintained by an  $H^+$  cotransport system. The correlation between phosphate uptake, the metabolic energy supply, and the intracellular phosphate level were studied.

## MATERIALS AND METHODS

**Plant Material.** Duckweed plants (*Lemna gibba* L., strain G1, and *Lemna paucicostata* Hegelm., strain 6746, obtained from the *Lemna* collection of Prof. Kandeler, Wien) were grown axenically on short days as described (27). The nutrient solution (17) contained 3.96 mM  $KNO_3$ , 5.47 mM  $CaCl_2$ , 1.22 mM  $MgSO_4$ , 1.47 mM  $KH_2PO_4$ , 18  $\mu M$  Fe-EDTA, 1.5  $\mu M$   $MnCl_2$ , 8.1  $\mu M$   $H_3BO_3$ , 0.5  $\mu M$   $Na_2MoO_4$ , and 29.2 mM sucrose (pH 4.8). Plants are termed high- $pd$  plants when they are characterized by an electrical membrane potential difference between the vacuole and the medium ( $pd$ ) of  $-200$  to  $-280$  mv in the dark, measured in  $1 \times SSC$  [1 mM KCl, 1 mM  $Ca(NO_3)_2$ , 0.25 mM  $MgSO_4$ , 0.95 mM  $NaH_2PO_4$ , buffered to pH 5.7 (9)]. They have an ATP pool of 70 to 105 nmol  $g^{-1}$  fresh weight, and an endogenous respiration rate of 4 to 9  $\mu mol O_2 g^{-1}$  fresh weight  $h^{-1}$  (14, 27). For phosphate starvation, the plants were kept in a phosphate-free culture medium ( $-P$ ) for 12 days. Prior to the experiments, the plants were transferred to  $1 \times SSC$  without phosphate for 5 h.

Low- $pd$  plants were obtained by growth for 8 days without sucrose under long day conditions. These plants are characterized by a  $pd$  of  $-90$  mv in the dark in  $1 \times SSC$ , a low ATP level of about 23 nmol  $g^{-1}$  fresh weight, and an endogenous respiration rate of less than 2  $\mu mol O_2 g^{-1}$  fresh weight  $h^{-1}$  (14, 27). In contrast to high- $pd$  plants, the  $pd$  of these plants was not affected by  $CN^-$  or transiently changed by glucose and glycine. The  $pd$  of low- $pd$  plants increased upon addition of high concentrations (50 mM) of glucose or upon illumination.

**Electrophysiological Measurements.** The fronds were mounted in a vertical Plexiglas chamber modified after Etherton and Higinbotham (6). The chamber was perfused with  $1 \times SSC \pm 1$  mM  $KH_2PO_4$  or with  $CaSO_4$  solution  $\pm 1$  mM  $NaH_2PO_4$  (pH 5.6 or 8) at a rate of 10 ml  $min^{-1}$ . Measurements of  $pd$  were performed by means of Ag/AgCl electrodes in glass micropipettes, containing 3 M KCl, inserted into the plants;  $pd$  was recorded by an electrometer amplifier (Keithley 604) and a chart recorder. Details were described previously (14).

**Phosphate Uptake.** Experiments were carried out as described for hexose uptake (27). The incubation solution ( $1 \times SSC$ ) was buffered to pH 5.6 with 5 mM Hepes. Irradiance was 140  $w m^{-2}$  and temperature was 25 C. Phosphate was labeled with  $^{32}P$  (Radiochemical Centre, Amersham, United Kingdom). After incubation, the plants were washed with unlabeled incubation solution

<sup>1</sup> This work was supported by a grant of the Deutsche Forschungsgemeinschaft (to U. L.) and by National Science Foundation Grant PCM 77-25575 (to A. N.).

<sup>2</sup> Dedicated to the memory of Noe Higinbotham, our friend and teacher in electrophysiology.

<sup>3</sup> Permanent address: Department of Plant Pathology, University of Missouri-Columbia, 108 Waters Hall, Columbia, MO 65211.

<sup>4</sup> Abbreviations:  $\Delta\mu H^+$ , proton electrochemical potential difference;  $pd$ , electrical membrane potential difference;  $c_o$ , external concentration;  $G$ , Gibbs free energy.

for 15 min at 0 C.  $^{32}\text{P}$  content and endogenous phosphate fractions were measured as reported earlier (26). One hundred mg fresh weight were used per sample.

## RESULTS

**Energy Dependence of Membrane Potential and of Phosphate Transport.** If energy-dependent phosphate transport depends on an actively maintained pd and if only the energy-dependent component of transport is accompanied by protons, phosphate influx must be significantly higher into high-pd plants than into low-pd plants.

High rates of phosphate transport into phosphate-starved duckweeds were correlated with a high pd. The high pd ( $-220$  mv) of duckweeds grown on sucrose was maintained in the dark and was not further increased by light (Table I). These high-pd and phosphate-starved plants attained also the maximum phosphate transport rate already in the dark (Table I,  $-P$  +sucrose plants) with only little additional stimulation by light. Phosphate transport rates of plants grown without sucrose showed a light stimulation of 140 to 230% of the dark rates (Table I,  $-$ sucrose + and  $-P$  plants).

The rate of phosphate influx into low-pd plants at pH 5.6 at an external concentration ( $c_o$ ) of  $50 \mu\text{M}$   $\text{H}_2\text{PO}_4^-$  (Table II) was only 20% of that of the high-pd plants (Table I,  $-$ sucrose  $-P$  plants, dark).

**Membrane Potential Changes during Phosphate Transport.** At the onset of phosphate transport, the pd of high-pd plants decreased by 50 to 66 mv within 70 s (Figs. 1 and 5) but spontaneously recovered to the original value within 7 min, even in the presence of 1 mM phosphate. Comparison of kinetic data of uptake with concentration-dependent depolarization of the membrane showed that, during glucose and glycine transport in *Lemna*, only the energy-dependent component of solute influx caused transient changes of pd (7, 14, 15, 27). Energy-dependent phosphate uptake in *Lemna* and phosphate-induced pd changes are saturated already at the low concentrations of 50 to  $100 \mu\text{M}$  phosphate (C. I. Ullrich-Eberius, unpublished data). Therefore, influx rates at  $c_o = 50 \mu\text{M}$  are compared here with transient pd changes at completely saturating conditions, i.e. at  $c_o = 1$  mM phosphate. Influx rates at  $c_o = 1$  mM phosphate (shown for comparison in Tables I and II) contain a component of influx, which caused no further change in pd.

To rule out cation effects on pd in experiments with  $\text{KH}_2\text{PO}_4$  (Fig. 5), external  $\text{K}^+$  concentration was kept constant throughout the experiments. KCl of the perfusion solution was substituted by

equimolar  $\text{KH}_2\text{PO}_4$ . In the experiments with  $\text{NaH}_2\text{PO}_4$  (Fig. 1), addition of 1 mM NaCl without phosphate to 0.5 mM  $\text{CaSO}_4$  solution had no effect on pd (Fig. 1D). In uptake experiments, substitution of 5 mM  $\text{Na}^+$  for  $\text{K}^+$  at pH 5 and at pH 8 had no effect on phosphate uptake either: at pH 5,  $\text{Na}^+$  and  $\text{K}^+$  were 994 and 1,111 nmol phosphate  $\text{g}^{-1}$  fresh weight  $\text{h}^{-1}$ , respectively; at pH 8,  $\text{Na}^+$  and  $\text{K}^+$  were 301 and 290 nmol phosphate  $\text{g}^{-1}$  fresh weight  $\text{h}^{-1}$ , respectively.

The low pd of  $-119$  mv at pH 5.6, which still contained a small active electrogenic component of about  $-30$  mv, decreased by only 18 mv during phosphate influx, i.e. 27% of the decrease in the high-pd control (Fig. 2A). Correspondingly, in plants with a medium pd in the dark ( $-150$  mv), phosphate transport caused only intermediate transient depolarization (30 mv, Fig. 3A). Light stimulated repolarization, thus resulting in a smaller depolarization (20 mv, Fig. 3B). In such carbon- and phosphate-starved plants, preillumination for 1 h further enhanced repolarization of the membrane after phosphate-induced depolarization and, thus, the amplitude of depolarization was reduced to only 10 mv (Fig. 3C). In contrast, phosphate uptake was enhanced by light (Fig. 4). Also, the hyperpolarization of the membrane upon sudden cessation of phosphate transport was smaller in the light and after prolonged preillumination in comparison to dark (Fig. 3). This is probably due to faster regulation of intracellular pH by light-enhanced metabolism. Thus, in the light, the smaller phosphate-induced depolarization cannot be explained as an inhibition but, rather, by an activation of the  $\text{H}^+$ -extrusion pump. This interpretation might be confirmed by the fact that fusicoccin, known to stimulate the  $\text{H}^+$ -extruding ATPase at the plasmalemma, caused the same phenomena on pd and on glucose and glycine transport in *Lemna gibba* (1) as light did in the present experiments.

**Effect of pH.** Carrier-mediated  $\text{H}^+$ -solute co-transport is supposed to depend on the extracellular pH in three ways. First, the activity of the carrier as a protein has its specific pH optimum of reaction with its specific substrate. Second,  $\text{H}^+$  cotransport should increase with the steepness of the pH gradient across the membrane. Third, the  $\text{H}^+$ -efflux pump is supposed to be more efficient when the pH gradient is small.

Phosphate uptake into *Lemna* had its optimum at pH 6. With increasing pH, the uptake decreased, in the dark as in the light, according to the decreasing concentration of monovalent  $\text{H}_2\text{PO}_4^-$  by dissociation of phosphate (Fig. 4). The pd remained constant between pH 5.6 and 8 in high-pd plants (Fig. 5). Corresponding to the reduced uptake rate at pH 8 (34%), the membrane was much less depolarized at the onset of phosphate transport (39%) compared with the depolarization at pH 5.6 (Fig. 5).

Table I. Effect of Phosphate and Sucrose Starvation

High-pd *L. gibba* were phosphate- and sucrose-starved for 12 days. The effects were measured on the intracellular phosphate level ( $c_i$ ), on phosphate uptake at  $c_o = 50 \mu\text{M}$  and 1 mM  $\text{NaH}_2\text{PO}_4$ , and on pd at pH 5.6 in the light (L) and in the dark (D). Values are means  $\pm$  SE. Numbers in parentheses signify number of experiments.

High-pd Plants Treatment	$c_i$	Phosphate Uptake				pd	
		$c_o = 50 \mu\text{M}$		$c_o = 1 \text{ mM}$		D	L
		D	L	D	L		
	$\text{mM PO}_4^{3-}$	$\text{nmol H}_2\text{PO}_4^- \text{ g}^{-1} \text{ fresh wt h}^{-1}$				$\text{mv}$	
+Sucrose +P	$41 \pm 1$ (36)	$44 \pm 2$ (8)	$53 \pm 3$ (8)	$450 \pm 25$ (4)	$500 \pm 19$ (4)	$-221 \pm 6$ (11)	
+Sucrose -P	$4 \pm 0.2$ (5)	$2,069 \pm 43$ (6)	$2,292 \pm 52$ (6)	$2,200 \pm 65$ (4)	$2,600 \pm 60$ (4)	$-226 \pm 5$ (16)	
-Sucrose +P	$26 \pm 0.4$ (17)	$10 \pm 1$ (8)	$24 \pm 1$ (8)			$-227 \pm 2$ (102)	$-220 \pm 4$ (15)
-Sucrose -P	$4 \pm 0.3$ (20)	$1,403 \pm 24$ (16)	$1,986 \pm 52$ (11)			$-221 \pm 3$ (5)	$-225 \pm 3$ (4)

Table II. Effect of pH on Pd and on Phosphate Uptake in Low-pd *L. gibba* in Dark

Five-min incubation at  $c_o = 50 \mu\text{M}$  or  $1 \text{ mM}$   $\text{NaH}_2\text{PO}_4$  in  $1\text{X } -\text{P}$  solution,  $1 \text{ mM}$  HEPES buffered with NaOH to pH 5.6, 7.0, or 8.0. Phosphate-starved plants were grown without sucrose for 12 days in long days. Values are means  $\pm$  SE and numbers in parentheses signify number of experiments.

Low-pd Plants	Pd mv	Phosphate Uptake $\text{nmol H}_2\text{PO}_4^- \text{ g}^{-1} \text{ fresh wt h}^{-1}$	
		$c_o = 50 \mu\text{M}$	$c_o = 1 \text{ mM}$
pH 5.6	$-106 \pm 4$ (5)	$267 \pm 13$ (6)	$533 \pm 6$ (3)
pH 7.0	$-122 \pm 8$ (5)		
pH 8.0	$-183 \pm 5$ (5)	$123 \pm 8$ (6)	$389 \pm 5$ (3)

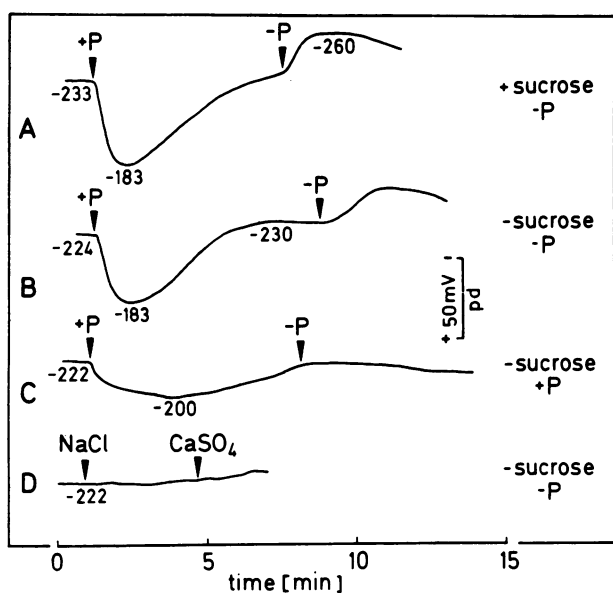


FIG. 1. Depolarization of the plasmalemma of high-pd *L. gibba* by  $1 \text{ mM}$   $\text{NaH}_2\text{PO}_4$  at pH 5.6 in  $0.5 \text{ mM}$   $\text{CaSO}_4$ , in the dark. Phosphate-starved ( $-\text{P}$ ) plants were kept on  $0.5 \text{ mM}$   $\text{CaSO}_4$  for 24 h prior to the measurements (A, B, and D). A, plants constantly grown on  $29 \text{ mM}$  sucrose; B, plants grown without sucrose for 12 days; C, plants grown on  $1.47 \text{ mM}$  phosphate, kept on  $0.5 \text{ mM}$   $\text{CaSO}_4$  for 8 h prior to the experiments; D, effect of  $1 \text{ mM}$  NaCl on pd in the presence of  $0.5 \text{ mM}$   $\text{CaSO}_4$  (2 to 10 replications each).

By contrast, in low-pd plants, a decrease of the external  $\text{H}^+$  concentration from pH 5.4 to 8 resulted, after some lag, in an increase of pd by  $-77 \text{ mV}$  (Fig. 2B). As shown in Table II, the main pH-dependent pd change occurred between pH 7 and 8 (about  $-60 \text{ mV}$ ) and was not reversible (Fig. 2B). This pH effect must be due to a stimulation of the  $\text{H}^+$ -efflux pump because, according to the Nernst potential for  $\text{H}^+$  fluxes, even at pH 8, the outwardly directed  $\text{H}^+$  flux requires energy. In these plants,  $1 \text{ mM}$  phosphate caused no depolarization at pH 8 but a slight hyperpolarization (Fig. 2C), and phosphate influx was only 40% of the rate which was already reduced by high pH in high-pd plants (Table II).

**Effect of Phosphate Starvation on Phosphate Transport.** When phosphate was omitted from the nutrient solution, the endogenous phosphate pool decreased to 80% of the control within only 24 h (Fig. 6). After 12 days, the phosphate pool had decreased to 7% of the +phosphate control. Both species investigated, *Lemma paucicostata* (Fig. 6) and *Lemma gibba* (Table I), showed very similar

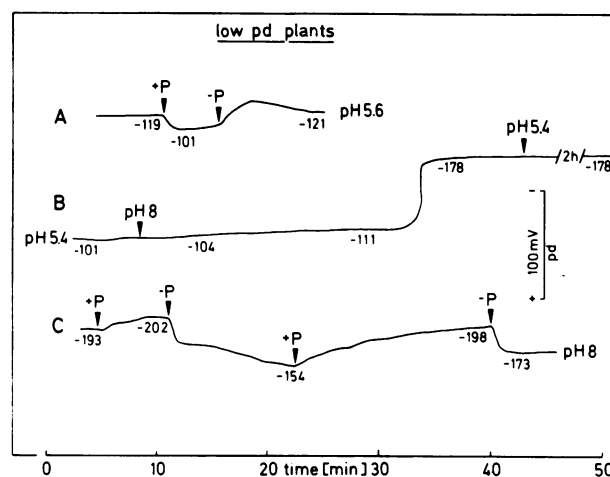


FIG. 2. Membrane potential changes of low-pd *L. gibba* by addition of  $1 \text{ mM}$   $\text{KH}_2\text{PO}_4$  in the dark to  $1 \times 1 \text{ SSC}/-\text{phosphate}$ . Phosphate-starved ( $-\text{P}$ ) plants were grown without sucrose for 12 days in long days. A, effect of phosphate at pH 5.6; B, effect of extracellular pH change from 5.4 to 8.0 on pd; C, effect of phosphate at pH 8 (three to six replications each).

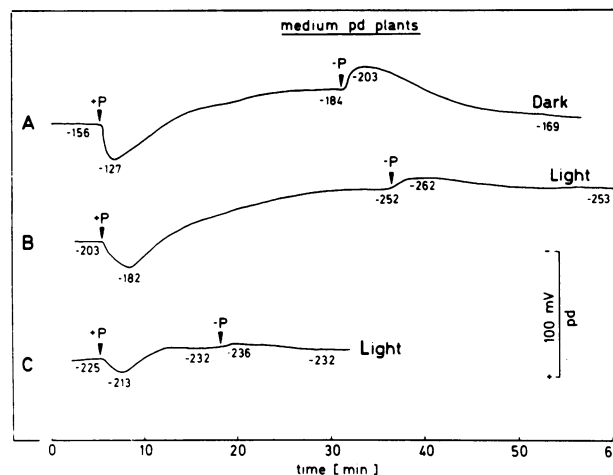


FIG. 3. Membrane potential changes of medium-pd *L. gibba*: depolarization by addition and hyperpolarization by removal of  $1 \text{ mM}$   $\text{KH}_2\text{PO}_4$  at pH 5.6. Phosphate-starved ( $-\text{P}$ ) plants were kept without sucrose for 7 days in long days. A, effects in the dark; B, effects in the light after 20 min preillumination; C, effects in the light after 1 h preillumination (four replications each).

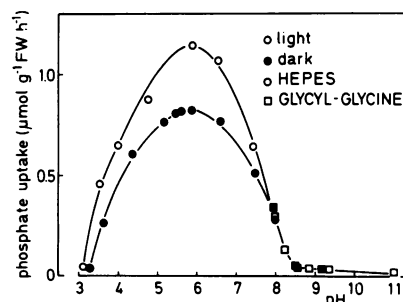


FIG. 4. pH-dependence of phosphate uptake in *L. gibba* in the light and in the dark;  $c_o = 50 \mu\text{M}$   $\text{KH}_2\text{PO}_4$ ; 10 min incubation. Plants phosphate-starved for 8 days (cf. Fig. 7); data are mean values of six experiments.

response to phosphate starvation. *L. paucicostata* also maintains a pd very similar to that of *L. gibba*, i.e.  $-200 \text{ mV}$  in the dark (12). The phosphate pool of *Lemma* is mainly in the acid-soluble

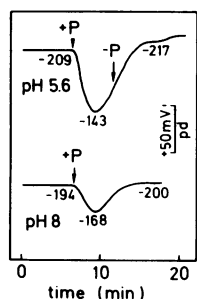


FIG. 5. Effect of pH on the phosphate-induced membrane depolarization in *L. gibba* in  $1\times/-P$  solution buffered with 1 mM Hepes-NaOH to pH 5.6 or 8.0; 1 mM  $\text{KH}_2\text{PO}_4$  was buffered with NaOH to pH 5.6 or 8.0; plants were grown on sucrose/ $-P$  for 12 days (10 and 3 replications each).

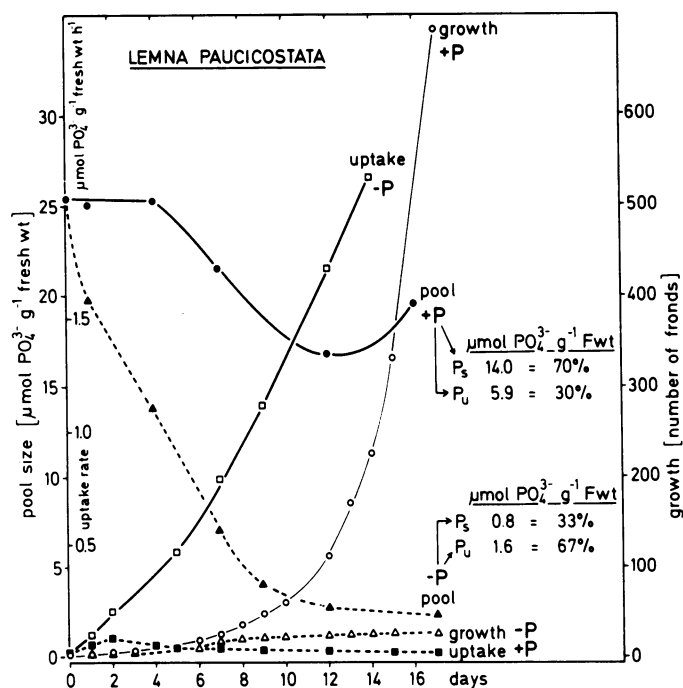


FIG. 6. Effect of phosphate starvation of *L. paucicostata* on the intracellular phosphate level (+P,  $c_o = 1.47$  mM  $\text{KH}_2\text{PO}_4$ ), on the phosphate uptake rate ( $c_o = 50$   $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ; incubation 60 min), on the growth rate, and on the phosphate distribution in two fractions during 17 days in long days. Plants were grown without sucrose. Mean values of  $n =$  six experiments.  $P_s$ , trichloroacetic acid-soluble fraction;  $P_u$ , trichloroacetic acid-insoluble fraction.

fraction (70%), containing Pi and only 3 to 5% organic phosphate compounds (Fig. 6). Phosphate starvation mainly caused a decrease of the Pi pool, down to 6% of the control (Fig. 6). Plant growth stopped completely within 7 days of phosphate starvation (Fig. 6).

During the same time, when the endogenous phosphate level decreased to one-tenth of the control, the uptake rate continuously increased to even more than 200 times that of +phosphate plants (Fig. 6). In accordance with the low rates of phosphate uptake in +phosphate plants, the membrane depolarization by 1 mM phosphate (kept for only 8 h on phosphate-free  $\text{CaSO}_4$  solution) was much smaller (33% on average) as compared with  $-P$  plants (Fig. 1C).

## DISCUSSION

**Evidence for  $\text{H}^+$ -Phosphate Cotransport.** *Lemna* plants maintain a high pd in the dark of  $-220$  mv as an average and an

intracellular phosphate concentration of 4 to 40 mM, and they accumulate phosphate at a rate between 9 and 2,300  $\text{nmol H}_2\text{PO}_4^- \text{g}^{-1}$  fresh weight  $\text{h}^{-1}$  at  $c_o = 50$   $\mu\text{M}$  phosphate, depending on the culture conditions (Table I). An accumulation factor can be calculated for plants permanently cultured at  $c_o = 1.47$  mM phosphate. As about 70% of the total intracellular phosphate is soluble Pi (Fig. 6), the intracellular phosphate level is 28.6 mM in +sucrose plants (Table I). The difference between the actively maintained pd measured and the Nernst potential for monovalent phosphate ( $\text{pd}_{\text{Pi}} = +76$  mv) shows that the driving force on passive phosphate fluxes in *Lemna* would be directed outward ( $\Delta\text{pd}_{\text{Pi}} = -296$  mv). Therefore, phosphate must be actively transported against the electrochemical gradient.

As during uptake of glucose (14), glycine (7, 15), and nitrate (15) in *Lemna*, pd transiently decreased also at the onset of active phosphate transport (Figs. 1 and 5), but only if pd was actively maintained at about  $-220$  mv.  $\text{Na}^+$  (1 mM) had no effect on pd or on phosphate influx. Phosphate-induced depolarization and phosphate influx rates seemed to be correlated: both were smaller at pH 8 than at pH 5.6, both larger in  $-P$  than in +phosphate plants (Figs. 1, 4, and 5; Table I). Since during  $\text{H}_2\text{PO}_4^-$  transport the membrane was depolarized in spite of its negative charge, more than one positive charge must accompany the anion. It is suggested that, in analogy to glucose uptake, protons are carried into *Lemna* cells during active phosphate transport along the  $\text{H}^+$  gradient. Only phosphate influx in low-energy plants hyperpolarized the membrane (Figs. 2C and 3, A and B) in a manner similar to that of *Trifolium* and *Helianthus* plants (3, 4). Measurements of extracellular pH changes in small volumes during phosphate uptake were not successful, probably due to the buffering properties of phosphate.

**Energy Requirement for Phosphate Transport.** If the driving force for phosphate transport and accumulation in *Lemna* is the electrochemical proton gradient ( $\Delta\mu\text{H}^+$ ), this gradient must thermodynamically account for the uptake rates measured between pH 3 and 8.5 (Fig. 4). In this pH range, due to the dissociation, both ionic species, monovalent and divalent phosphate, occur in the extracellular medium. For transport of monovalent phosphate, an energy requirement of  $\Delta G = 28.6$   $\text{kJ mol}^{-1}$  and for transport of divalent phosphate, an energy requirement of  $\Delta G = 50$   $\text{kJ mol}^{-1}$  can be calculated from the accumulation ratio under steady-state equilibrium conditions (see above). The pd of  $-220$  mv average (Table I) is equivalent to an energy of  $\Delta G = 21.3$   $\text{kJ mol}^{-1}$ . Thus, the electrical component of  $\Delta\mu\text{H}^+$  alone would not be sufficient to maintain  $\text{H}^+$ -phosphate cotransport. Additional energy could be contributed by the chemical  $\text{H}^+$  gradient across the membrane ( $\Delta\text{pH}$ ). At a pH of 4.8, as in the culture medium,  $\Delta\text{pH}$  is assumed to be 2.6 units (21). Then  $\Delta\mu\text{H}^+$  could account for 36  $\text{kJ mol}^{-1}$ , sufficient for the transport of protons required to neutralize the monovalent phosphate and to charge the carrier positively. On the supposition of  $\text{H}^+$  cotransport, transport of divalent phosphate appears to be thermodynamically impossible. The pH optimum for phosphate uptake in *Lemna* is at pH 6, which is energetically less favorable for  $\text{H}^+$  cotransport. However, at pH 6,  $\Delta\mu\text{H}^+$  is equivalent to  $\Delta G = 30$   $\text{kJ mol}^{-1}$  and, therefore, still sufficient for phosphate transport. Above pH 6,  $\text{H}^+$ -phosphate cotransport would be thermodynamically improbable. But energy-dependent phosphate uptake occurs even at pH 8. In addition, at the onset of transport, there is still a depolarization of 20% of that observed at pH 5.6, i.e. an excess of positive charges must be transported into the cell, together with phosphate. If phosphate uptake is mediated by an  $\text{H}^+$  cotransport mechanism, uptake at pH 8 in the bulk of the medium can only be explained by the model of Kotyk (11). His hypothesis postulates an  $\text{H}^+$ -ATPase, providing the carrier with a local pH gradient. The observation that *Lemna* plants, incubated at pH 8, acidify even the bulk of the external solution rather rapidly seems to support this assumption.

From the results presented here, it is concluded that, in higher plants, like in bacteria and fungi (2, 5, 8, 18), phosphate transport is mediated by  $H^+$  cotransport. It depends on the maintenance of  $\Delta\mu H^+$  by intracellular energy supply as well as on the carrier activity regulated by the intracellular phosphate level.

*Acknowledgment*—We thank Mrs. Luise Neher very much for her expert technical assistance.

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