# Effect of Aluminum on the Uptake and Metabolism of Phosphorus by Barley Seedlings[1]

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*Summary.* The uptake of $P^{32}$ and its incorporation into phosphorylated compounds was examined in the roots of barley seedlings which had been pretreated with aluminum.

The rate at which phosphorus increased in Al-roots was greater than in controls, especially during the first 15 minutes of incubation. It was shown that the increased phosphorus in Al-roots was $P_i$ and that it was almost completely exchangeable. Similar increases over controls were found when root segments were incubated in phosphorus solutions containing $10^{-3}$ M DNP and at low temperature. The increased $P_i$ in Al-roots did not result in an increase in the total amount of phosphorus incorporated into phosphorylated compounds.

Aluminum treatment markedly decreased the incorporation of $P^{32}$ into sugar phosphates but increased the pool size of ATP and other nucleotide triphosphates present in the roots. The specific activities of $P^{32}$ in ATP in Al-roots and controls were similar indicating that the rates of ATP synthesis were similar in each case.

Preliminary investigations showed that aluminum citrate inhibited both purified yeast hexokinase and phosphorylated sugar production by crude mitochondrial extracts from barley roots.

The results suggest that there are 2 reactions between aluminum and phosphorus: 1) at the cell surface or in the free space which results in the fixation of phosphate by an adsorption-precipitation reaction; 2) within the cell, possibly within the mitochondria, which results in a marked decrease in the rate of sugar phosphorylation, probably effected by the inhibition of hexokinase. The evidence does not support the view that aluminum enhances phosphorus uptake or that the superficial reaction between aluminum and phosphate interferes with phosphorus transport.

Cationic aluminum is known to affect the growth of plants in 2 ways: firstly through an inhibition of cell division in roots (6, 20), and secondly by reducing the transport of phosphorus from the roots to the shoots. This reduction in phosphorus transport results in the development of phosphorus deficiency symptoms in the shoots of barley plants (9). Both aluminum and phosphorus accumulate in barley roots and it has been suggested that some form of internal precipitation of aluminum phosphate accounts for the observed reduction in phosphorus transport to the shoots (22, 23). Recently, several authors (14, 15) have described experiments in which roots treated with aluminum appear to have an enhanced phosphorus uptake. It is possible that these results, and those of Wright (22) may be explained by an interaction between aluminum and phosphorus at the root or cell surface rather than by an effect on phosphorus uptake. Another possibility is interference with phosphorus transport within the plant. In this paper both these possibilities are examined and a mechanism suggested by which aluminum might cause phosphorus fixation in the root.

## Materials and Methods

*Material.* Barley seeds, *Hordeum vulgare* L., var. Proctor (Marsters Seeds, King's Lynn, England), were soaked in distilled water for 6 hours and then germinated on trays of polythene mesh which rested on the surface of a culture medium. The seedlings were grown at 25° in an aerated, phosphate-free culture solution (pH 4.5) in which the following compounds were supplied in mM concentrations: KCl 0.5, $CaCl_2$ 2.5, $NH_4NO_3$ 1.0, $MgSO_4$ 0.25, ferric citrate 0.04 and trace element supplement (1). All the chemicals were of analytical reagent grade. During this period and in subsequent experimental operations the plants were illuminated continuously by eight 40 w color matching fluorescent tubes (Compton) giving a radiant flux of 2.7 kiloergs $cm^{-2} \cdot sec^{-1}$ at the level of the plants. Three days after soaking half of the plants were placed in a culture medium similar to that described above but containing in addition either 0.5 or 1.0 mM aluminum [as $Al_2(SO_4)_3$].

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while the remaining plants were returned to fresh culture solution without addition of aluminum to act as controls. After a further 1 or 2 days the plants were removed from the solutions, the roots washed thoroughly with distilled water and placed in radioactive phosphorus incubating solutions. Following incubation the roots were washed in 5 changes of distilled water.

*Extraction and Counting Procedures.* Total radioactive phosphorus in roots and shoots was determined on 0.1 N HCl extracts of material ashed at 500° for 24 hours. There was no detectable losses of phosphorus during this process as compared with wet ashing in a mixture of concentrated nitric, sulphuric and perchloric acids.

Acid soluble phosphate esters and $P_i$ were extracted according to the scheme described by Bieleski and Young (5). This scheme, which involves the killing and extraction of tissue in a methanol-chloroform-formic acid (MCF) solvent, has proved most satisfactory; the extracts have high ATP/ADP ratios, and the absence of methyl phosphate on the chromatograms indicates that phosphatase activity during the killing process was negligible (4).

RNA was hydrolyzed to nucleotide monophosphates by treatment of the residue from the MCF extraction with 0.3 N KOH. Total RNA was estimated by colorimetric determination of ribose by the method of Ogur and Rogen (13), using dilutions of adenosine to make a standard curve. The specific activity of $P^{32}$ in RNA was estimated by counting the radioactivity of mononucleotide spots on chromatograms.

*Chromatography.* Extracts were applied to Whatman 3 MM chromatography paper which had been previously treated with 1 % oxalic acid and then washed with double glass distilled water. A 2-dimensional separation of phosphate esters and nucleotides was effected using *n*-propanol-0.880 $NH_4OH$-water (6:3:1, v/v) as the first solvent, and *n*-propyl acetate-formic acid (90 %)-water (11:5:3 v/v) as the second. Ten ml of 10 $^{-1}$ M EDTA disodium salt were added to each liter of both these solvents. Papers were treated with the first solvent for 48 hours and with the second for 9 hours. The positions of radioactive spots were located by autoradiography (Ilford Industrial Xray film exposed for 48-96 hours). Spots were cut from the chromatograms and the radioactivity measured directly using an end-window Geiger Müller tube. Recovery of radioactivity in distinct spots was 85 to 95 % of that applied to the starting line, the remainder being accounted for by a diffuse area of phospholipid. The separation of individual sugar monophosphates was not very satisfactory and in the data below they are grouped together as sugar phosphate.

*Identification of Spots.* Markers were added singly to various MCF extracts and their position matched with auto radiograms. Nucleotides were detected by their absorption of UV radiation. Sugars and sugar phosphates were detected by spraying papers with *p*-anisidine reagent and heating at 105° for 5 minutes. Phosphate esters were detected by lightly spraying papers with 1 % ammonium molybdate reagent as described by Bandurski and Axelrod (2) and exposing the air-dried papers to UV radiation.

*Chemical Estimation of Phosphorus.* Phosphorus was determined spectrophotometrically using the method of Fiske and Subbarow (7) as modified by Bartlett (3). Potassium dihydrogen phosphate was used to prepare standard solutions containing 0.1 to 1.0 μmole $PO_4$.

## Results

All the experiments in this section have been repeated at least 3 times and the results presented below are from representative experiments.

*Uptake and Distribution of $P^{32}$ in Whole Plants of Barley.* Seedlings were treated with 0.5 mM aluminum sulfate for 48 hours; untreated plants served as controls. Both batches were then incubated in $10^{-4}$ M sodium dihydrogen phosphate plus 1 mc $P^{32}$/liter (carrier-free $KH_2P^{32}O_4$). Three batches of 10 plants were taken from both treatments after 1, 10, $10^2$ and $10^3$ minutes incubation in the phosphate medium and the dry weights of the shoots and roots in each batch determined before ashing at 500° for 24 hours.

Table I shows that in the aluminum-treated roots (subsequently referred to as Al-roots) there was a rapid initial increase of radioactivity in the first 10 minutes, resulting in a 4-fold increase over that in the control plants which had not been treated with aluminum. The differential established during this period was maintained and perhaps slightly increased during the remainder of the experiment. Thus, the contribution of this initial phase of rapid uptake to the total phosphorus in the roots decreased in significance as the experiment proceeded. In an experiment described below it was shown that most of the extra phosphorus in Al-roots was held in an exchangeable form and was therefore taken to be outside the cell membrane (8).

Table 1. *$P^{32}$ Incorporated into Dry Plant Material*

Values are in counts per sec per mg. Mean of 3 replicate samples. Al-plants were pretreated with 0.5 mM aluminum sulfate for 48 hours. Control plants grown in a similar culture medium without added aluminum. O signifies radioactivity not significantly greater than background.

| Incubation time (Min) | | Roots Mean | ± | SD | Shoots Mean | ± | SD |
|---|---|---|---|---|---|---|---|
| 1 | Al | 30.3 | ± | 2.87 | 0 | | |
| | Control | 7.3 | ± | 1.90 | 0 | | |
| 10 | Al | 75.0 | ± | 9.60 | 0 | | |
| | Control | 33.8 | ± | 1.60 | 0 | | |
| $10^2$ | Al | 283.5 | ± | 34.93 | 3.8 | ± | 0.64 |
| | Control | 247.3 | ± | 32.71 | 6.5 | ± | 2.05 |
| $10^3$ | Al | 693.4 | ± | 57.80 | 89.5 | ± | 19.23 |
| | Control | 626.4 | ± | 53.36 | 136.2 | ± | 12.66 |

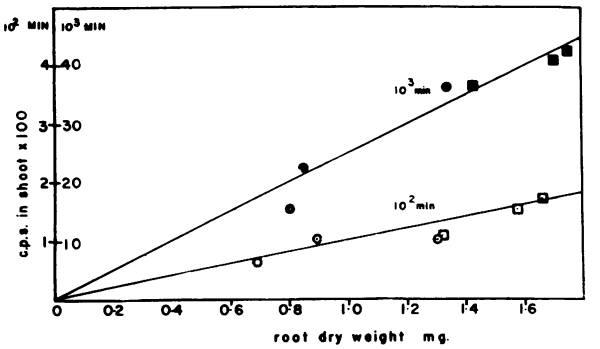


FIG. 1. Total radioactivity in the shoots of barley plants as a function of root weight. ●, ○, Roots pretreated with 0.5 mM aluminum sulphate for 48 hours in a culture medium with pH 4.4. ■, □, Control roots raised in a similar medium without aluminum.

The radioactivity in the shoots did not become significant until the plants had been incubated for 100 minutes. In the shoots of plants treated with aluminum there was approximately 40 % less $P^{32}$/g of tissue than in the controls after $10^2$ and $10^3$ minutes incubation. At first sight this result might be interpreted as a considerable reduction in phosphorus transport. There is, however, another possible explanation. The roots of Al-plants were smaller than those of control plants while the shoots of plants in both treatments were of similar weight. The smaller roots were caused by the inhibitory effect of aluminum on cell division (6, 20). In figure 1, where the mean root weight of plants in each replicate is plotted against the total radioactivity in the shoots, it is clear that there is a linear relationship which suggests that the amount of phosphorus transported to the shoot per unit weight of root is the same in both control and Al-plants. Differences in shoot concentration result from different shoot root ratios.

*Incorporation of $P^{32}$ into Phosphorylated Compounds in Whole Barley Roots.* In an experiment similar to the one described above, roots were incubated in a medium containing $5 \times 10^{-5}$ M $NaH_2PO_4$ plus 1 mc $P^{32}$/liter. Samples were taken after 1, 5, 20 and 100 minutes and the phosphorylated compounds in the roots extracted in MCF.

Increases in the total radioactivity in MCF extracts from Al-treated and control roots follow a closely similar pattern to that observed in the gross uptake of $P^{32}$ in experiment 1 (table II).

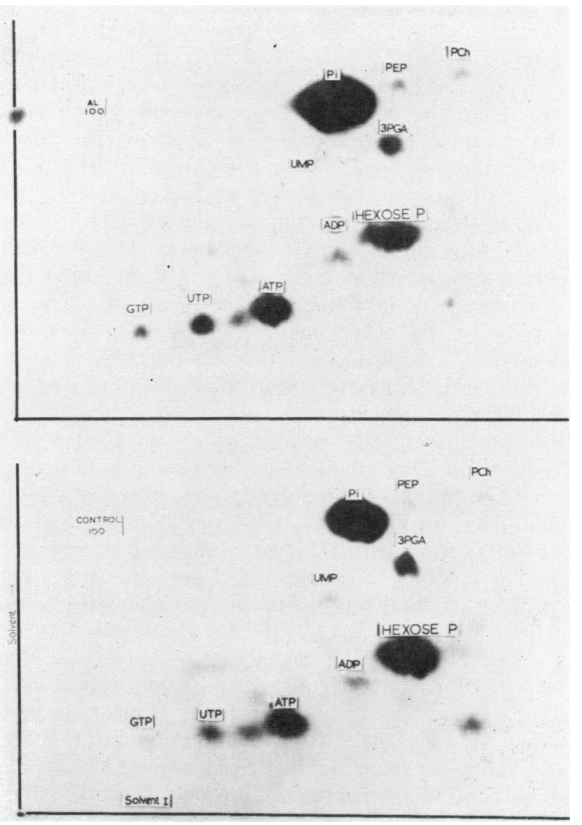


FIG. 2. Autoradiographs of chromatograms of phosphorylated compounds from MCF extracts of barley roots. Roots incubated in $5 \times 10^{-5}$ M $NaH_2PO_4$ plus 1 mc $P^{32}$/liter for 100 minutes. Al-100 signifies roots pretreated with 0.5 mM aluminum sulfate for 48 hours in a culture medium with pH 4.4. Control 100 signifies control roots raised in a similar medium without aluminum.

Table II. *Incorporation of $P^{32}$ into Phosphorylated Compounds Extracted in MCF*

Values are in counts per sec per root. Mean of 2 replicates. Aluminum treated plants were kept in a culture solution (pH 4.4) containing mM aluminum sulfate for 24 hours. Control plants were raised in a similar medium without aluminum sulfate added. ..., signifies no spot detected on autoradiograms.

| | 1 Min | | 5 Min | | 20 Min | | 100 Min | |
|---|---|---|---|---|---|---|---|---|
| Spot | Control | Aluminum | Control | Aluminum | Control | Aluminum | Control | Aluminum |
| GTP | ... | ... | 2.4 | 1.5 | 3.7 | 6.9 | 11.2 | 22.0 |
| GDP | ... | ... | ... | 1.3 | 2.7 | 3.1 | 7.4 | 7.2 |
| UTP | ... | ... | 2.4 | 2.2 | 6.6 | 10.4 | 9.7 | 14.2 |
| ATP | 2.7 | 1.0 | 9.7 | 6.7 | 19.2 | 29.3 | 36.8 | 56.0 |
| ADP | 1.2 | ... | 2.7 | 2.0 | 4.2 | 6.2 | 11.4 | 9.2 |
| Sugar P | 1.5 | ... | 26.6 | 3.6 | 57.5 | 20.5 | 160.8 | 66.0 |
| 3 P-Glycerate | ... | ... | 4.7 | 1.2 | 6.4 | 3.1 | 33.3 | 15.6 |
| $P_i$ | 35.0 | 390.7 | 83.8 | 541.7 | 237.3 | 804.0 | 524.2 | 1303.6 |
| Total | 40.4 | 391.7 | 133.4 | 560.2 | 337.6 | 883.5 | 794.6 | 1490.8 |

Chromatographic separation of these extracts showed that the increased radioactivity in the Al-roots was principally due to a marked increase in $P_i$, (fig 2). The increased $P_i$ in Al-roots did not result in any striking increase in the total incorporation of phosphorus into esterified compounds, and in this respect it may be regarded as being unavailable for metabolism. Aluminum had an effect on the distribution of $P^{32}$ in phosphorylated compounds (table II). In all samples there was a marked reduction in the counts incorporated into sugar phosphates and a slight increase in ATP in the 20 and 100-minute samples. Both these results are consistent with the idea that aluminum directly or indirectly inhibits the phosphorylation of glucose and fructose which are the principal components of the sugar phosphates described here (12). This suggests that aluminum may inhibit hexokinase activity. The radioactivity in other nucleotide triphosphates was greater in Al-roots. Whether the increase was due to more rapid turnover or to increased pool size was examined in experiment 3.

*Uptake and Incorporation of $P^{32}$ by Barley Root Segments.* Three-day-old barley plants were divided into 2 groups. The first group was grown in a phosphate-free medium (pH 4.4) for 48 hours and the second treated with 0.5 mM aluminum sulfate in a similar medium. Samples of 1-cm segments were cut 3 mm behind the tips of the roots and washed in aerated distilled water at 25° for 2 hours before use in experiments. The segments were divided into batches of 5 and then placed in sample tubes which were kept at 25° in a water bath. Each tube contained 10 ml of $2 \times 10^{-5}$ M $KH_2PO_4$ plus 1 $\mu$c $P^{32}$ and other nutrients as described above. The tubes were aerated intermittently through fine glass jets. At each sampling time 6 tubes from each treatment were taken at random. The segments from 3 of these replicates were washed thoroughly with distilled water and placed on albumin/glycerol-coated planchets. The segments in the 3 remaining replicates were quickly washed with cold distilled water and placed in 50 ml of $10^{-2}$ M $KH_2PO_4$ at 0° in an ice bath; they were removed after 30 minutes, washed and placed on planchets. The segments on the planchets were dried under an infrared lamp and their radioactivity determined directly using an end-window Geiger-Müller tube. The albumin/glycerol coating on the planchets prevented the segments curling up during drying.

The increase in the total radioactivity of the control and Al-segments showed a similar pattern to that of whole roots (table III). In the first few minutes the radioactivity in Al-segments increased much more rapidly than in the controls, and thereafter a slightly increased rate was maintained. The rapidity of the initial phase in Al-segments suggests that there was some surface reaction between phosphorus and aluminum which was quite independent of the normal uptake mechanism.

When exchangeable phosphorus in the Al-segments was removed by the treatment with $10^{-2}$ M $KH_2PO_4$ it was evident that the amount of phosphorus within the cells was similar to that in the cells of control segments.

As a final test of the hypothesis, that the aluminum/phosphorus interaction is external, phosphorus uptake by control and Al-segments was examined at low temperature (1°) and in the presence of $10^{-3}$ M dinitrophenol (DNP). The segments were prepared as above but were pretreated for 10 minutes with either $10^{-3}$ M DNP or aerated distilled water held at 1° in an icebath.

The incubation medium was the same as that described above with the exception that in one treatment dinitrophenol was added to bring the final concentration to $10^{-3}$ M.

The results in table IV show that there is an appreciable increase in the radioactivity of Al-segments over a period of 1 hour in the presence of DNP and at low temperature, whereas there was little increase in the control segments. The phosphate bound in the presence of DNP and at low temperature was nearly all exchangeable.

Larger samples of segments were taken after 20 minutes' incubation in $10^{-5}$ M $NaH_2PO_4$ plus 2 mc $P^{32}$/liter and the distribution of $P^{32}$ in the compounds extracted by MCF and 0.3 N KOH were examined.

Table III. *Incorporation of Phosphorus by Barley Root Segments*

Values are in mμmoles P per g (fr wt). Mean of 3 replicates with standard deviation. Aluminum treatment was with 0.5 mM aluminum sulfate in a culture medium with pH 4.4 for 48 hours. Control plants raised in a similar medium with no aluminum added.

| Sample | Treatment | Total P | Nonexchangeable P | Exchangeable P (by deduction) |
|---|---|---|---|---|
| 2 Min | Control | 16 ± 2 | 14 ± 1.8 | 2 |
| | Aluminum | 97 ± 8.5 | 18 ± 1.8 | 79 |
| 15 Min | Control | 103 ± 15.1 | 92 ± 11.5 | 11 |
| | Aluminum | 172 ± 13.2 | 91 ± 18.3 | 81 |
| 60 Min | Control | 402 ± 47.0 | 399 ± 51.9 | 3 |
| | Aluminum | 588 ± 76.3 | 365 ± 48.5 | 223 |
| 120 Min | Control | 520 ± 62.1 | 478 ± 35.6 | 42 |
| | Aluminum | 897 ± 24.5 | 459 ± 88.3 | 438 |
| 240 Min | Control | 990 ± 168.1 | 786 ± 101.6 | 204 |
| | Aluminum | 1517 ± 168.9 | 831 ± 113.9 | 686 |

Table IV. *Incorporation of Phosphorus by Barley Root Segments in the Presence of DNP and at Low Temperature*

Values are in mμmoles P per g (fr wt). Mean of 3 replicates with standard deviation. Al-plants were pretreated with 0.5 mM aluminum sulfate for 48 hours. Control plants were grown in a similar medium with no aluminum added.

| | Sample | Treatment | Total P | Nonexchangeable P | Exchangeable P (by deduction) |
|---|---|---|---|---|---|
| | 2 Min | Control | 8.7 ± 1.2 | 0 | 8.7 |
| | | Aluminum | 28.2 ± 4.1 | 0 | 28.0 |
| $10^{-3}$ | 15 Min | Control | 29.6 ± 4.7 | 1.0 ± 0.3 | 28.6 |
| DNP | | Aluminum | 62.1 ± 5.9 | 2.2 ± 0.4 | 59.9 |
| 25° | 30 Min | Control | 33.4 ± 5.1 | 5.0 ± 0.2 | 28.4 |
| | | Aluminum | 98.4 ± 7.2 | 3.0 ± 0.1 | 95.4 |
| | 60 Min | Control | 34.3 ± 3.6 | 2.2 ± 0.1 | 32.1 |
| | | Aluminum | 129.6 ± 14.7 | 7.1 ± 0.2 | 122.5 |
| | 2 Min | Control | 0.6 ± 0.1 | 0.2 ... | 0.4 |
| | | Aluminum | 32.7 ± 5.1 | 0.7 ± 0.2 | 32.0 |
| | 15 Min | Control | 1.0 ± 0.2 | 0.6 ± 0.1 | 0.4 |
| 1° | | Aluminum | 84.4 ± 1.3 | 0.5 ± 0.3 | 83.9 |
| | 30 Min | Control | 8.1 ± 1.3 | 1.9 ± 0.2 | 6.2 |
| | | Aluminum | 130.9 ± 12.6 | 2.6 ± 0.5 | 151.6 |
| | 60 Min | Control | 12.0 ± 2.4 | 2.9 ± 0.3 | 9.1 |
| | | Aluminum | 179.3 ± 18.2 | 2.5 ± 0.3 | 176.8 |

Table V. *Incorporation of $P^{32}$ into Phosphorylated Compounds in Root Segments*

Values are in counts per sec per g (fr wt). Aluminum-treated roots grown in 0.5 mM aluminum sulfate for 48 hours. Controls were grown without aluminum. Roots incubated in $10^{-5}$ M $NaH_2PO_4$ plus 2 mc $P^{32}$/liter for 20 minutes.

| | Control | Aluminum treated |
|---|---|---|
| GTP | 32 | 62 |
| UTP | 54 | 133 |
| ATP | 169 | 277 |
| ADP | 39 | 62 |
| Sugar P | 933 | 620 |
| 3 P-glycerate | 143 | 111 |
| P-Enolpyruvate | 53 | 49 |
| P-Choline | 40 | 42 |
| $P_i$ | 2939 | 22,604 |

Table VI. *Pool Size and Specific Activity of $P^{32}$ in Phosphorylated Compounds in Aluminum-Treated and Control Root Segments*

| | Pool size (Total P) μmoles per g fr wt | | Specific activity Counts per sec per μmole P | |
|---|---|---|---|---|
| | Control | Aluminum | Control | Aluminum |
| ATP | 0.323 | 0.711 | 275 | 339 |
| ADP | 0.172 | 0.197 | 222 | 211 |
| UTP | 0.210 | 0.807 | 140 | 100 |
| Sugar P | 2.764 | 2.391 | 109 | 114 |

In MCF extracts the distribution of $P^{32}$ in nucleotides and other esters was similar to that found in whole roots, but the increased incorporation into nucleotide triphosphates in Al-segments was more strongly marked than in experiment 2 (table V). The pool size of nucleotides and sugar phosphates was estimated by cutting out spots on chromatograms and determining total phosphorus. The specific activity of $P^{32}$ in nucleotides and sugar (table VI) shows that the increased incorporation of $P^{32}$ into ATP and UTP in the Al-segments was correlated with an increased pool size of these compounds. The pool size of sugar phosphates was markedly reduced in Al-segments although specific activity of $P^{32}$ in the pool was similar in both treatments. These results lead to the conclusion that aluminum, presumably present in the mitochondria or at their surface, markedly reduces the rate of phosphorylation of sugars although there is a large pool of ATP present.

The total RNA in segments from both treatments was similar, but the specific activity of RNA-$P^{32}$ was somewhat lower in segments from aluminum treated roots (table VII).

Preliminary experiments have shown that aluminum citrate inhibits yeast hexokinase and also glucose phosphorylation by crude extracts from barley root mitochondria. Figure 3 shows that concentrations of aluminum citrate in the range $5 \times 10^{-3}$ M to $5 \times 10^{-2}$ M result in 16 to 50 % inhibition of purified yeast hexokinase. Similar concentrations of sodium citrate did not inhibit. Aluminum citrate was used because of the very low solubility of uncomplexed aluminum at the pH of the enzyme assay.

A fuller account of the inhibition of hexokinase

Table VII. *Incorporation of $P^{32}$ in RNA in Segments of Barley Roots*

| Treatment | Total RNA μg per g fr wt | Specific activity $P^{32}$ Counts per sec per μg RNA-P |
|---|---|---|
| Control | 1181 | 1.52 |
| Aluminum | 1385 | 0.93 |

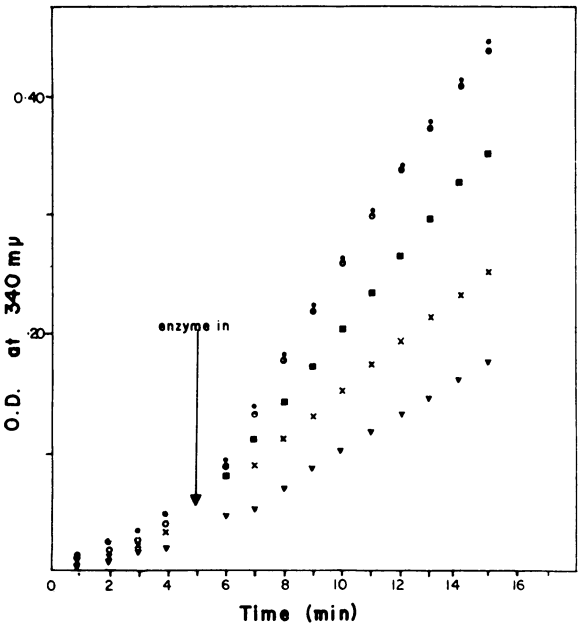


FIG. 3 The effect of aluminum citrate on the activity of yeast hexokinase. Assay modified from Kornberg's procedure for ATP assay (11). The activity of hexokinase is linked with the production of NADPH which is followed by an increase in extinction at 340 mμ. Standard conditions: Silica cells of 1 cm pathlength; temperature 27°; reactants, ATP 1.5 mM, glucose 5 mM, NADP 0.2 mM, $MgSO_4 \cdot 7H_2O$ $10^{-2}$ M, KCl $10^{-1}$ M, Tris-HCl (pH 7.5) 0.07 M and 2 μmole units glucose-6-phosphate dehydrogenase (Sigma). 1 μg purified yeast hexokinase (Sigma) added after 5 minutes. Aluminum or sodium citrate added to reactants before the addition of hexokinase. Total volume of reactants 2.0 ml. ○, Control, no aluminum citrate; ●, $10^{-2}$ M sodium citrate; ■, $5 \times 10^{-3}$ M aluminum citrate; ×, $10^{-2}$ M aluminum citrate; ▼, $5 \times 10^{-2}$ M aluminum citrate.

by aluminum citrate and a possible mechanism for it is in preparation.

## Discussion

It is clear from the foregoing results that there are 2 interactions between aluminum and phosphate in barley roots, one at the cell surface or in the free space which is nonmetabolic and the other inside the cell, perhaps in the mitochondria, which affects the phosphorylation of hexose sugars.

The rapid initial increase in the phosphorus content of barley roots which have been pretreated with aluminum is due to inorganic phosphate. A similar finding has been reported recently by Rorison (18) working with the excised roots of *Onobrychis sativa* (sainfoin). The persistence of the rapid initial phase in the presence of dinitrophenol and low temperature and the almost complete exchangeability of the phosphorus thus incorporated is strong evidence for the location of this $P_i$ at the cell surface. The distribution of aluminum in the cellular components of roots has shown that in roots treated with mM aluminum sulfate 85 to 95 % of the total aluminum is located in the cell wall fraction (Clarkson, unpublished data). With aluminum thus located, the processes involved in the aluminum-phosphorus interaction might be regarded as an extension of the processes of phosphorus fixation which have been described in soils. Hsu and Rennie (10) have shown that during the hydrolysis of the ion $Al^{3+}$ the last hydroxyl group, added to $Al(OH)_2^+$ is unstable and remains freely exchangeable with $H_2PO_4^-$ in solution, resulting in the formation of $Al(OH)_2H_2PO_4$. Thus, aluminum hydroxide may fix phosphorus by an adsorption-precipitation reaction. At the pH used in the present experiments (pH 4.4) the predominant aluminum ion species is $Al(OH)_2^+$ (16); it seems probable that hydroxyl ions at the root and cell surface may result in the precipitation of $Al(OH)_3$. Aluminum hydroxide surfaces are usually positively charged below pH 5, and often below pH 6.5 to 6.7 (17). If this is so, then an adsorption-precipitation reaction similar to the one described above may occur. Some evidence to support the view that $Al(OH)_3$ is present at the root surface is provided by the observation that only a small proportion of the aluminum in whole roots or in cell wall preparations is exchangeable with other cations. When plants are growing in a soil where aluminum and phosphorus are both present it is possible to envisage this adsorption-precipitation reaction as a continuous process which would effectively reduce the amount of phosphorus available for transport to the shoots and entry into the metabolic pools in the root. The precipitation of aluminum phosphate from solutions is slow even in supersaturated conditions, but it has been shown by Hsu and Rennie (10) that surfaces upon which aluminum is adsorbed greatly accelerate the reaction. In the present work, where aluminum and phosphate are presented to the plant separately, the reduced phosphorus concentration in the shoots is not likely to be accounted for by this precipitation, since, as the incubation period was extended to $10^3$ minutes, the aluminum/phosphate reaction would have reached or approached equilibrium. The results also show that the total phosphorus actually within the cells (i.e. nonexchangeable) was similar in both aluminum-treated and control roots. In the absence of any agreement about the movement of phosphorus across the root and into the stele it is difficult to decide whether the disturbances in phosphate metabolism, after active uptake, result in a disturbance of phosphorus transport. It was pointed out above that the amount of phosphorus transported per unit weight of root was the same in plants from both treatments, and that the concentration differences in the shoots resulted from different shoot/root ratios in aluminum-treated and control plants. A more rigorous examination of this point, which has been largely ignored in work hitherto, is clearly desirable. Wright and Donahue (23) report an experiment in which phosphorus trans-

port to the shoots of plants pretreated for 4 weeks with 0.4 mM aluminum sulfate was compared with control plants over an incubation period of only 4 hours. The aluminum-treated plants had, in the authors' words, very few roots, much stunted and brittle. No weights are given by which the roots in the 2 treatments can be compared, but autoradiographs of the plants suggest that the root size in the aluminum-treated plants is less than one-tenth of that in the controls grown without aluminum, while the shoots were about one-third of the size. In these circumstances it is hardly surprising to find that the concentration of phosphate in the shoots of aluminum-treated plants was lower. Wright and Donahue's conclusion that the reduction in transport is due to internal precipitation has been questioned before (21) and the evidence in the present work suggests that the precipitation is neither internal nor of much significance in phosphorus uptake and transport.

The surface reaction between phosphorus and aluminum is perhaps a more likely explanation of the increased phosphorus content of snap-bean roots (14) when aluminum was included in the incubation medium, than an enhancement of phosphorus uptake. At least some of the data presented by Randall and Vose (15) may be interpreted similarly. These authors did find, however, that certain aluminum treatments increased the transport of phosphorus to the shoots of perennial rye grass, and suggest that at certain aluminum concentrations the phosphorus transport mechanism may be enhanced.

A small proportion of the total aluminum found in roots appears to be inside the cells and the data above show that this fraction has an inhibitory effect on the phosphorylation of hexose sugars. The decrease in the pool size of hexose phosphate is coupled with an increased pool of ATP and other nucleotide triphosphates suggest that aluminum either inhibits hexokinase or combines with the substrate to make it unavailable. The preliminary in vitro experiments with purified yeast hexokinase and crude extracts from barley root mitochondria confirm that the presence of aluminum as aluminum citrate inhibits the enzyme at concentrations similar to those of other metallic inhibitors (19). These studies also suggest that treatment of the enzyme with aluminum prior to its assay does not result in a marked inhibition of enzyme activity.

The increased pool size of UTP and the increased incorporation of $P^{32}$ into GTP in Al-roots may result from equilibration with ATP or from a reduction in the rate of RNA synthesis. The latter suggestion is supported by a reduction in the specific activity of phosphorus in RNA in Al-roots. It should be remembered, however, that during the 48-hour treatment of the roots prior to incubation in phosphate those roots treated with aluminum stopped growing, whilst those in the control medium did not. The number of cells dividing in root apices declines sharply 6 to 10 hours after treatment with mM aluminum sulfate. Thus, after 48 hours' incubation in aluminum sulfate the cells in the 1-cm segment behind the root tip will be older than those from the similar segment in control roots. My own observation is that cell elongation and differentiation are not inhibited by aluminum treatment. The extent to which this age difference may affect RNA synthesis is unknown. A similar slight reduction in the specific activity in a metabolically labile fraction of DNA in barley roots has been reported (20).

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