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# Nitrate Uptake by Dark-grown Corn Seedlings

## SOME CHARACTERISTICS OF APPARENT INDUCTION[1]

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### ABSTRACT

**Five- or six-day old seedlings of corn (*Zea mays* L.) were exposed to 0.25 mM $Ca(NO_3)_2$, 1.0 mM sodium 2-[N-morpholino]-ethanesulfonate, 5 μg Mo per liter and 50 μg of chloramphenicol per ml at pH 6. Nitrate uptake was determined from depletion of the ambient solution. The pattern of nitrate uptake was characterized, after the first 20 minutes, by a low rate which increased steadily to a maximal rate by 3 to 4 hours. Transfer of nitrate to the xylem did not totally account for the increase. Development of the maximal accelerated rate did not occur at 3 C with excised roots nor with seedlings whose endosperm had been removed. Use of $CaCl_2$ rather than $Ca(NO_3)_2$ resulted in a linear rate of chloride uptake during the first 4 hours, and chloride uptake was not as restricted by endosperm removal as was nitrate uptake.**

**Nitrite pretreatments or the addition of cycloheximide (2 μg $ml^{-1}$), puromycin (400 μg $ml^{-1}$) and 6-methylpurine (0.5 mM) restricted maximal development of the accelerated nitrate uptake rate. Actinomycin D (20 μg $ml^{-1}$) inhibited the rate only after about three hours exposure. The RNA and protein synthesis inhibitors also restricted nitrate reductase induction in the apical segments of the root tissue. The data suggest that development of the maximal accelerated rate of nitrate uptake depended upon continuous protein synthesis, and the hypothesis that synthesis of a specific nitrate transport protein must occur is advanced. But the alternative hypothesis, *i.e.*, that induction of nitrate reductase (and/or a consequence of the act of nitrate reduction) provided the required stimulus, remains tenable.**

A nitrate uptake pattern characterized by a slow initial rate and a subsequent accelerated rate occurred when wheat seedlings previously grown without nitrogen were first exposed to nitrate-containing solutions (23). Similar patterns were obtained with nitrate salts of potassium, sodium, magnesium, and calcium (24), although the presence of calcium tended to shorten the initial or lag phase (23). Unpublished studies of G. A. Thompkins, W. A. Jackson, and R. J. Volk reveal that prior exposure to ammonium ion did not eliminate the initial lag phase. The same pattern also has been observed with cotton and tobacco depleted of nitrate prior to the experimental period and with 10-day-old corn grown exclusively with ammonium ion as the nitrogen source (11). The latter observation contrasted to the nitrate uptake pattern of similar corn plants grown with nitrate in which case nitrate uptake did not exhibit the initial lag phase. A similar increase in nitrate uptake rate has been reported recently for tobacco cell suspension cultures (8) which had been grown previously with urea and in cultures which were nitrogen starved.

The present investigation with dark-grown corn seedlings was initiated to characterize more fully this nitrate uptake pattern by root tissue of higher plants. It is shown that a substance from the endosperm was essential for full development of the accelerated rate of nitrate uptake, and that this development was restricted by certain inhibitors of RNA and protein synthesis.

### MATERIALS AND METHODS

All experiments were conducted with corn hybrid (*Zea mays* L.) OH43 × B14, except that DeKalb XL45 hybrid was used to obtain part of the data of Figure 5. DeKalb XL45 and OH43 × B14 show similar patterns of nitrate uptake. Corn seeds were rinsed for 1 min in 5.25% NaOCl, placed in running tap water for 3 hr, rinsed again for 1 min in the NaOCl solution, rinsed thoroughly in deionized water, and about 150 seeds were spread on stainless steel screens (14 × 14 cm) over 4-liter containers of vigorously aerated 0.1 mM $CaSO_4$. The seeds were covered with one layer of filter paper kept moist by wick action. Germination took place at 25 C in darkness. Four days later the seedlings were removed, and all roots except the primary root were excised. The primary roots of approximately 100 seedlings were then threaded through a stainless steel screen into a 4-liter beaker containing 0.5 mM $(NH_4)_2SO_4$, 0.6 mM $K_2SO_4$, 0.4 mM $KH_2PO_4$, 1.6 mM $MgSO_4$, 0.8 mM $CaSO_4$, and 0.125 g of $CaCO_3$/l. Iron was supplied at 4 mg/l as monosodium hydrogen ferric diethylenetriaminepentaacetate, and the remaining trace elements were at one-fifth the concentration of Hoagland's solution (9). The containers were again placed in the dark germinator at 25 C, and the solutions were aerated vigorously.

After a further 24 or 48 hr, the etiolated seedlings were removed, and the roots were rinsed thoroughly in deionized water. The seedlings were then floated in trays on 0.5 mM $CaSO_4$ while unifrom seedlings were selected, assembled in groups of seven, and supported in plastic frames. Roots of each group were then blotted lightly with paper towels and placed

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at timed intervals in tubes (2.5 × 20 cm) containing the appropriate solutions. Except for the experiments reported in Figures 7 and 8, the etiolated shoots were excised approximately 3 cm above the seed immediately prior to placing the seedlings in the uptake solution. Solutions were aerated gently throughout the course of the experiment.

Exuding xylem sap was removed periodically by blotting or collected during the course of the experiment by capillary action with a small diameter plastic tube. In some experiments (Fig. 8 and 9), the endosperm also was excised immediately prior to placing the roots of the seedlings in the uptake solution. When excised roots alone were used (Fig. 7), they were excised 1 cm below the seed, and the upper ends were held together and out of the solution with nylon thread. The exuding sap from these roots was also removed periodically and not permitted to contaminate the uptake solution.

The standard uptake solution consisted of 0.50 mM nitrate as $Ca(NO_3)_2$, 1.0 mM Na 2-[N-morpholino]ethanesulfonate, 5 $\mu$g of Mo per liter (as $Na_2MoO_4$), and 50 $\mu$g of chloramphenicol (the D[−]threo-isomer) per ml at pH 6.0. The pattern of nitrate uptake was not affected by this concentration of chloramphenicol (Fig. 12), and it was routinely used to curtail bacterial contamination (10, 26, 32). For measurements of chloride uptake, 0.25 mM $CaCl_2$ was substituted for $Ca(NO_3)_2$. Usually 70 ml of uptake solution were employed per seven plants. The containers were held in a water bath at 30 C, and each treatment was replicated at least three times in each experiment. At precisely timed intervals after introduction of the roots into the solution. 0.5 ml of the solution was removed. Nitrate content of the ambient solution and the collected xylem sap was determined by the method of McNamara *et al.* (21). From the solution-analyses the cumulative uptake patterns shown in the figures were determined. In most experiments the uptake solutions were not replaced, so a progressive decrease in the ambient nitrate concentration occurred. In some instances the concentration was so depleted before the experiment was terminated that the nitrate uptake rate was obviously affected (Figs. 6, 8, and 10), in which case the mean nitrate uptake rates (shown in the inserts of the figures) do not include these abnormally low values observed during the final stages of the experiment. For the experiments shown in Figures 4 and 9, the ambient solutions were changed hourly so that progressive depletion was minimized. Chloride uptake was determined by removing aliquots from the ambient solution, acidifying, and titrating with $Hg(NO_3)_2$ using diphenylcarbazone as indicator. Because of the use of solution-depletion procedures, all uptake rates reported herein for both nitrate and chloride are net rates.

At the end of the experimental period, roots were rinsed 10 times in ice cold 0.5 mM $CaSO_4$, excised immediately below the seed, blotted uniformly, and weighed. All data are based on 1 g of fresh root weight and are the means of all treatment replications. Tissue nitrate concentrations were determined on aliquots after homogenization with 0.025 M $K_2HPO_4$, pH 8.6.

For extraction of nitrate and nitrite reductase, root segments were homogenized thoroughly with a glass Ten-Broeck homogenizer (approximately 2 min of vigorous grinding) with 0.025 M $K_2HPO_4$, 1mM $Na_2EDTA$, and 1 mM cysteine (final pH 8.5) using five volumes per gram of root tissue. After centrifuging at 29,000*g* for 15 min, the supernatant was used as enzyme source without further purification. Nitrate reductase was assayed as described by Hageman and Hucklesby (7). Nitrite reductase was assayed by the method of Joy and Hageman (13) using dithionite and methyl viologen as electron donors. Reactions were conducted at 30 C.

Oxygen uptake measurements were conducted by conventional Warburg manometry at 30 C. Solutions in the flasks were identical to those used in the uptake measurements, and triplicate measurements were made for each treatment.

Total soluble sugars were extracted with methanol-chloroform-$H_2O$ (13:4:3) and determined by the phenol-$H_2SO_4$ method.

## RESULTS

**Nitrate Uptake and Translocation Patterns.** Cumulative nitrate uptake data from two typical experiments are shown in Figures 1 and 2. Inserts show the mean rates of uptake during each measurement period plotted at the midpoint of the period. In most experiments (Fig. 1), nitrate uptake exhibited an adsorption shoulder during the first 20 min (19). This shoulder was not always noted (Fig. 2). Nevertheless, in all experiments the period immediately following the first 20 min was characterized by a low uptake rate (hereafter called the lag phase) which then increased to a maximal rate (hereafter called the accelerated phase). The latter was usually 5 to 6 $\mu$moles $g^{-1}$ $hr^{-1}$, was attained by the 3rd or 4th hr and remained relatively constant for at least the next 3 or 4 hr. The variable rates observed in the first 20 min among experiments did not influence the subsequent general pattern. Accordingly, the inserts of the subsequent figures do not include the values obtained during the first 20 min; the first point plotted is the uptake rate between 20 and 60 min. The increase to the accelerated rate occurred in spite of progressive depletion of the ambient solution from 0.5 mM to less than 0.1 mM. Exposing the roots to 3 C eliminated the increase in rate (Fig. 2).

Accumulation of nitrate in the xylem exudate also increased during the experimental period (Fig. 1), but it constituted a relatively small proportion of the nitrate taken up. In four

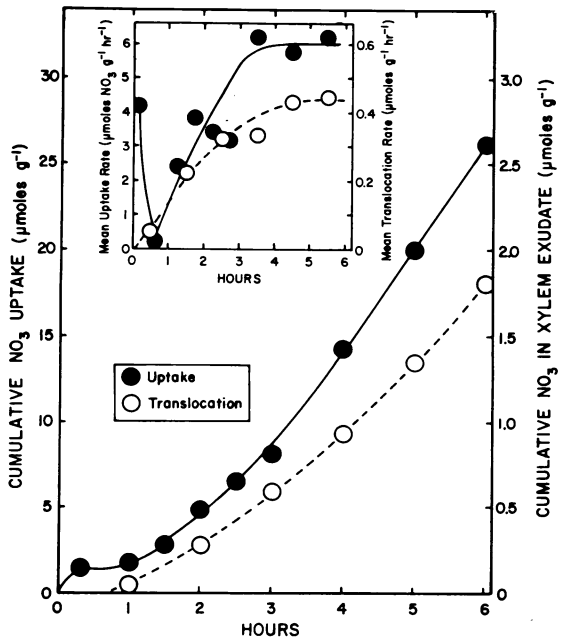


FIG. 1. Nitrate uptake and translocation of nitrate to exuding xylem sap of dark-grown corn seedlings. Shoots were excised immediately prior to placing the solutions in the uptake solution. Initial nitrate concentration of the aerated uptake solution was 0.5 mM as $Ca(NO_3)_2$ and also contained 1.0 mM Na 2-[N-morpholino]ethanesulfonate, pH 6, 5 $\mu$g Mo per liter and 50 $\mu$g chloramphenicol $ml^{-1}$. The solution was not changed during the experiment so the nitrate concentration progressively declined. Rates of uptake and translocation shown in the insert are plotted at the midpoint of each measurement period. Temperature was 30 C throughout.

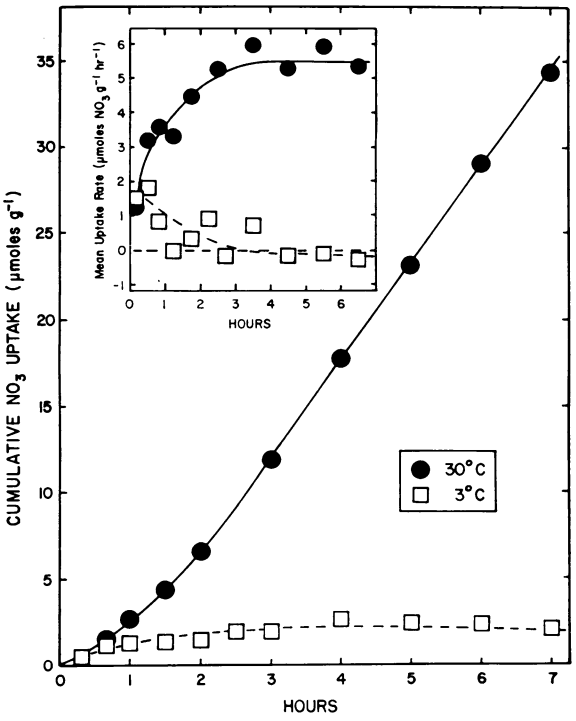


FIG. 2. Influence of temperature on nitrate uptake. Other conditions were as in Figure 1.

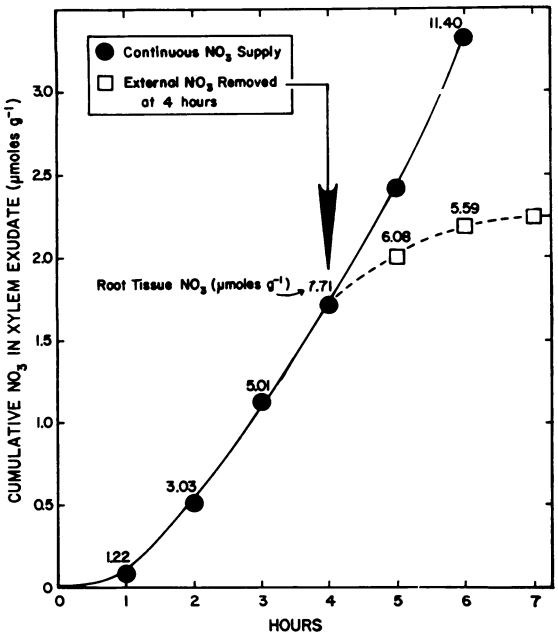


FIG. 3. Influence of presence of external nitrate on deposition of nitrate in exuding xylem sap. Values shown beside the plotted points are the nitrate concentrations of the total root tissue. Conditions were as in Figure 1.

separate experiments, nitrate recovered in the xylem sap ranged from 4 to 17% of total nitrate uptake, but deposition of nitrate in the exudate was nevertheless closely associated with the uptake process. When seedlings were transferred at 4 hr from the standard $Ca(NO_3)_2$ solution to a comparable one of $CaSO_4$, only 0.54 μmole nitrate $g^{-1}$ were translocated in the subsequent 3 hr, although the total root system contained 7.7 μmoles nitrate $g^{-1}$ at the time of transfer (Fig. 3).

**Chloride and Nitrate Uptake.** In contrast with nitrate uptake, chloride uptake from $CaCl_2$ was essentially linear during the first 5 hr and then declined (Fig. 4). During the accelerated phase nitrate uptake exceeded the earlier linear chloride uptake rate. Oxygen uptake rates for the apical 1.5 cm were 678 and 626 μl $O_2$ $g^{-1}$ $hr^{-1}$ after 3 hr of exposure to chloride and nitrate treatments, respectively; for 1.5 to 3.0-cm segments the values were 412 and 460 μl $O_2$ $g^{-1}$ $hr^{-1}$, respectively. A separate experiment employing the entire terminal 10.5-cm portion of roots gave values of 497 and 481 μl $O_2$ $g^{-1}$ $hr^{-1}$ for the chloride and nitrate treatments, respectively.

**Induction of Nitrate and Nitrite Reductases.** Prior to exposure to the nitrate solutions there was little nitrate reductase activity in the root tissue but nitrite reductase activity was substantial (Fig. 5, Table I). With DeKalb XL45, increased activity of both enzymes was detectable after 1 hr, and the increase was essentially linear thereafter (Fig. 5). A lower level of induction for OH43 × B14 was obtained in spite of a higher nitrate content in the 3.5-cm root segment. Removal of the external nitrate after 4 hr of exposure prevented any further increase in net nitrate reductase activity of OH43 × B15, even though the segments contained from 8.7 to 6.0 μmoles $NO_3^-$ $g^{-1}$ during the last 3 hr (Fig. 5).

**Nitrite Pretreatments.** Two hours exposure to 0.5 mM $NaNO_2$ failed to eliminate the lag period of nitrate uptake (Fig. 6). During the pretreatment, 5.5 μmoles $NO_2^-$ $g^{-1}$ had been absorbed and completely assimilated. A 2-hr pretreatment with 2.0 mM $NaNO_2$, during which time 12.4 μmoles $NO_2^-$ $g^{-1}$ were absorbed and assimilated, prolonged rather than shortened the duration of the lag period (data not presented). Nitrite pretreatment decreased the magnitude of the accelerated phase (Fig. 6), but there was no significant change in nitrate reductase of the root tips at the end of the experiments. Values for the 0- to 1.5-cm apical segments were 0.50 and 0.51 μmole $g^{-1}$ $hr^{-1}$ for the NaCl and $NaNO_2$ pretreatments, respectively for the experiment of Figure 6. The corresponding values for the 1.5- to 3.0-cm segments were 0.41 and 0.38 μmole $g^{-1}$ $hr^{-1}$.

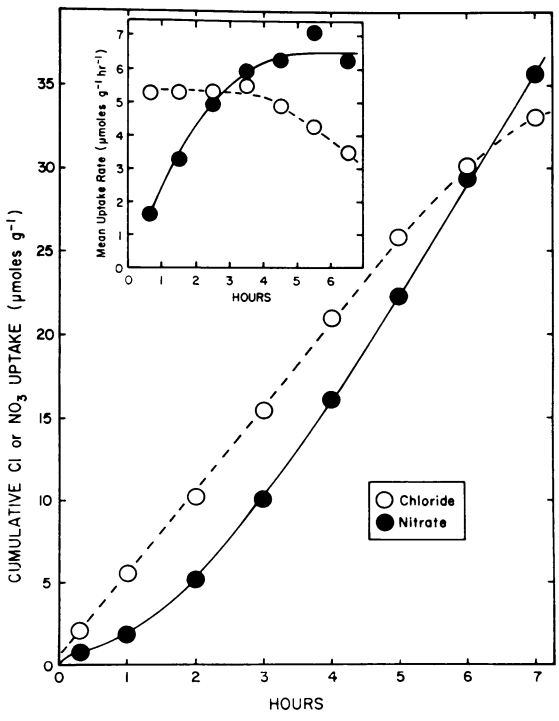


FIG. 4. Comparison of chloride and nitrate uptake from 0.5 mM solutions replaced hourly. Other conditions were as in Figure 1.

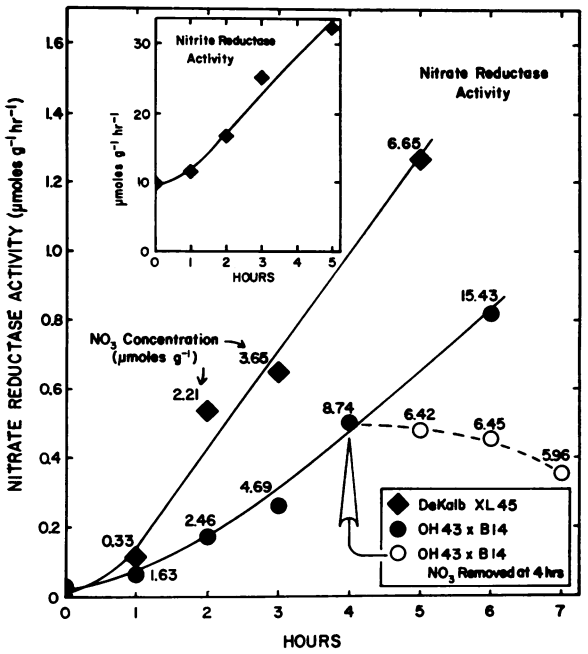


FIG. 5. Time course of induction of nitrate and nitrate reductases in the apical 3.5-cm segments of roots and the requirement for continual external nitrate supply on nitrate reductase induction. Values beside the plotted points are the nitrate concentrations of the apical 3.5-cm segments. Conditions were as in Figure 1.

Table I. *Influence of 5-Fluorouracil, Actinomycin D, and 6-Methylpurine on Nitrate and Nitrite Reductase Activities of Corn Roots*

Assays were made after 7-hr exposure to nitrate-containing solutions on plants whose nitrate uptake patterns are shown in Figure 10.

| Root Segment | Treatment | Nitrate Reductase | | Nitrite Reductase | |
|---|---|---|---|---|---|
| | | μmoles $g^{-1}$ $hr^{-1}$ | % control | μmoles $g^{-1}$ $hr^{-1}$ | % control |
| 0–1.5 cm | Initial | 0.07 | | 12.0 | |
| | Control | 0.52 | 100 | 21.5 | 100 |
| | 5-Flurouracil (2.5 mM) | 0.53 | 102 | 21.3 | 99 |
| | Actinomycin D (20 μg $ml^{-1}$) | 0.18 | 35 | 15.2 | 71 |
| | 6-Methylpurine (0.5 mM) | 0 | 0 | 11.7 | 54 |
| 1.5–3.0 cm | Initial | 0.02 | | 5.5 | |
| | Control | 0.18 | 100 | 16.9 | 100 |
| | 5-Flurouracil (2.5 mM) | 0.17 | 94 | 16.3 | 96 |
| | Actinomycin D (20 μg $ml^{-1}$) | 0.03 | 18 | 6.5 | 38 |
| | 6-Methylpurine (0.5 mM) | 0 | 0 | 6.2 | 37 |

**Tissue Requirements for Accelerated Nitrate Uptake.** Excised roots did not consistently exhibit the accelerated nitrate uptake rate and the rate declined noticeably after about the 3rd hr (Fig. 7). Removal of the endosperm, regardless of whether the shoot was excised or not, curtailed maximal development of the accelerated rate (Fig. 8). The decline in uptake rate in plants without endosperm did not occur as rapidly as it did with excised roots (Fig. 7). Chloride uptake was essentially unaffected by endosperm removal (Fig. 9). The somewhat lower maximal rate in intact plants compared to those with shoots removed (Fig. 8) has been detected in other experiments, although the apparent decline in rate noted in this experiment (after having reached a maximal rate) has not been consistently observed (Figure 7, experiment 1).

**RNA and Protein Synthesis Inhibitors.** At 2.5 mM, 5-flurouracil did not appreciably alter the nitrate uptake pattern (Fig. 10), whereas 6-methylpurine (0.5 mM) completely prevented development of the accelerated rate. Actinomycin D (20 μg $ml^{-1}$) was ineffective until the 3rd hr by which time it essentially prevented any further increase in the uptake rate (Fig.

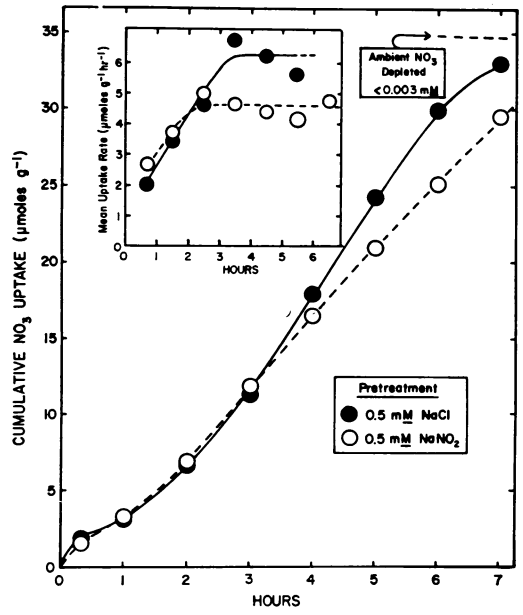


FIG. 6. Influence of 2-hr pretreatment with 0.5 mM $NaNO_2$ compared with NaCl on subsequent nitrate uptake. Other conditions were as in Figure 1.

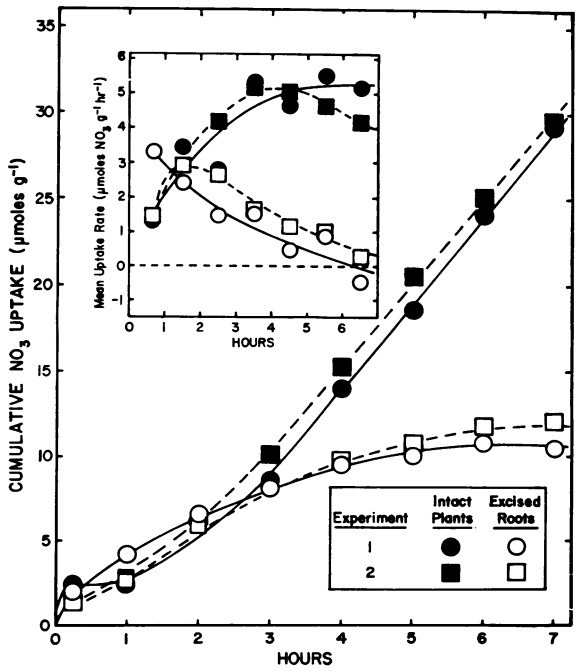


FIG. 7. Comparison of nitrate uptake by intact plants and excised roots. Other conditions were as in Figure 1.

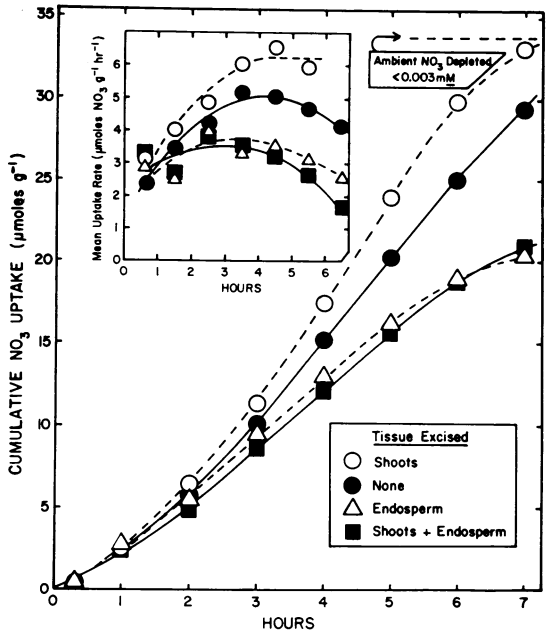


FIG. 8. Influence of tissue excision on nitrate uptake. Other conditions were as in Figure 1.

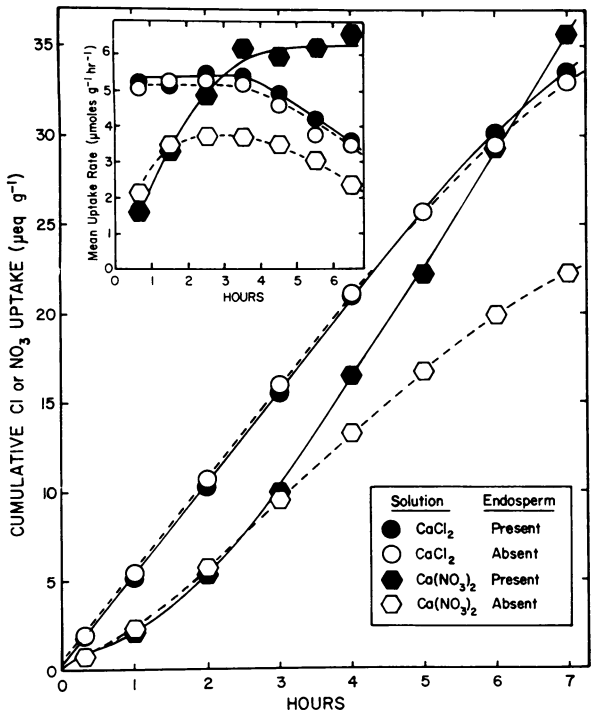


FIG. 9. Comparison of endosperm removal on nitrate and chloride uptake from 0.5 mM solutions replaced hourly. Other conditions were as in Figure 1.

10). When actinomycin D was added 2 hr after exposure to nitrate, a further 3 hr passed before the uptake rate was affected (Fig. 11). Initially the uptake solutions contained no potassium. Analyses at the termination of the experiment of Figure 10 showed that 0.2 to 0.4 μmole $K^+$ $g^{-1}$ had been released from the roots with no significant difference between treatments. Therefore suppression of the accelerated phase of nitrate uptake by actinomycin D and 6-methylpurine (Fig. 10) appeared not to result from an altered membrane permeability.

Nitrate and nitrite reductase activities in the apical 0 to 1.5 and 1.5 to 3.0 cm regions of the roots at the end of the experiment of Figure 10 were not significantly affected by 5-flurouracil (Table I). Induction of both enzymes were severely restricted by actinomycin D and was essentially eliminated by 6-methylpurine. A relatively greater influence of actinomycin D on induction of nitrate reductase than on nitrate uptake or nitrate translocation to the xylem was confirmed by the results of the experiment in Figure 11 (Table II).

Cycloheximide at 2 μg $ml^{-1}$ (Fig. 12) and puromycin at 400 μg $ml^{-1}$ (Fig. 13) largely prevented development of the accelerated rate of nitrate uptake. Chloramphenicol at 50 μg

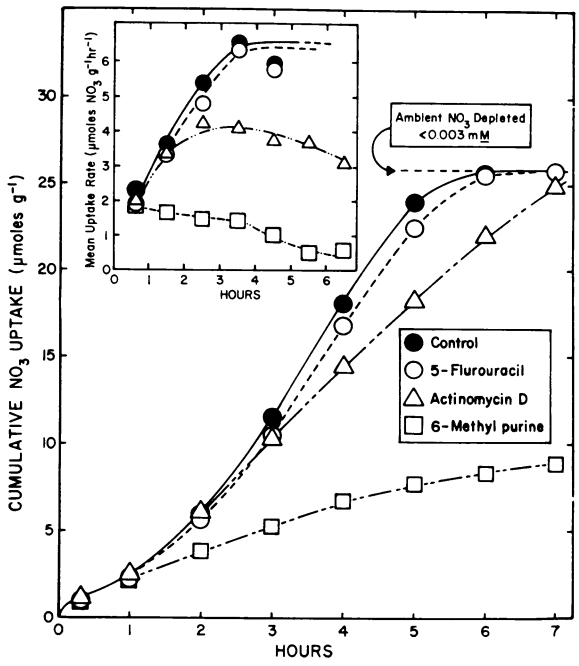


FIG. 10. Influence of 5-flurouracil (2.5 mM), actinomycin D (20 μg $ml^{-1}$) and 6-methylpurine (0.5 mM) on nitrate uptake. Other conditions were as in Figure 1.

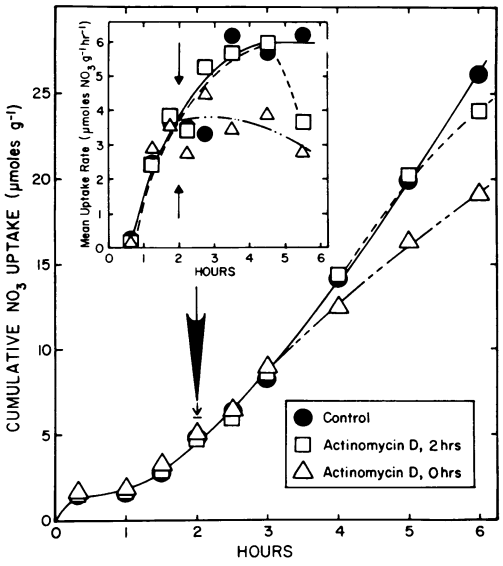


FIG. 11. Influence on nitrate uptake of actinomycin D (20 μg $ml^{-1}$) applied at the outset and after 2-hr exposure to nitrate. Other conditions were as in Figure 1.

$ml^{-1}$ was without effect (Fig. 12). The influence of puromycin was evident within the 1st hr, whether applied at the outset or 2 hr after the plants had first been exposed to nitrate (Fig. 13). Measurement of $O_2$ uptake by the terminal 10.5 cm of roots excised after 3-hr exposure to puromycin gave an average of 431 $\mu l$ $O_2$ $g^{-1}$ $hr^{-1}$ which was 90% of the rate observed with roots of plants in nitrate solutions without puromycin. After 3 hr of puromycin treatment, nitrate uptake was less than 50% of the control (Fig. 13). Nitrate reductase activity in the root tissue was drastically reduced by puromycin as was the amount of nitrate deposited in the xylem (Table III).

## DISCUSSION

**The Nitrate Uptake Pattern.** After the first 20 min, a general uptake pattern, characterized by a lag phase which increased steadily to an accelerated phase by 3 to 4 hr, was observed in all experiments. The accelerated phase was completely elimi-

Table II. *Influence of Actinomycin D on Nitrate Uptake, Nitrate Translocation, and Nitrate Reductase Activity of Corn Roots*

Values were obtained after 6-hr exposure to nitrate-containing solution from plants whose nitrate uptake patterns are shown in Figure 11. Nitrate reductase assays were made on apical 3.5-cm root segments. Values in parentheses are per cent of the treatment without actinomycin D.

| | Actinomycin D (20 $\mu g$ $ml^{-1}$) Treatment | | |
|---|---|---|---|
| | None | At start | After 2 hr |
| Nitrate uptake ($\mu$moles $g^{-1}$) | 26.2 | 19.2 (73) | 24.1 (92) |
| Nitrate in xylem exudate ($\mu$moles $g^{-1}$) | 1.80 | 1.56 (87) | 1.87 (104) |
| % Translocation | 6.9 | 8.1 (117) | 7.8 (113) |
| Nitrate reductase ($\mu$moles $g^{-1}$ $hr^{-1}$) | 0.91 | 0.14 (15) | 0.46 (51) |

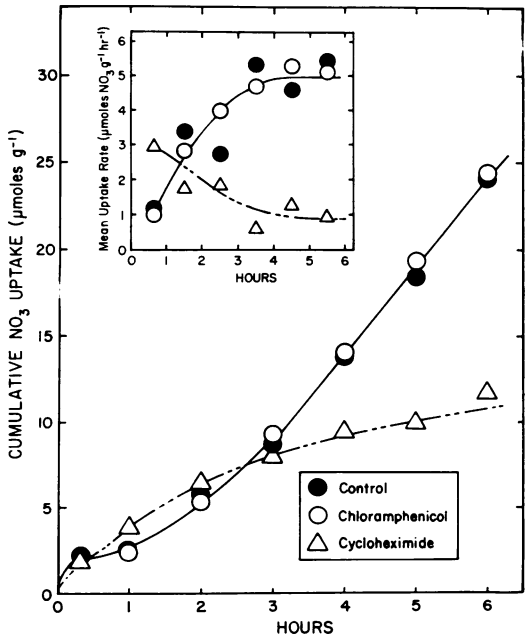


FIG. 12. Influence of chloramphenicol (50 $\mu g$ $ml^{-1}$) and cycloheximide (2 $\mu g$ $ml^{-1}$) on nitrate uptake. Intact plants were used; other conditions were as in Figure 1.

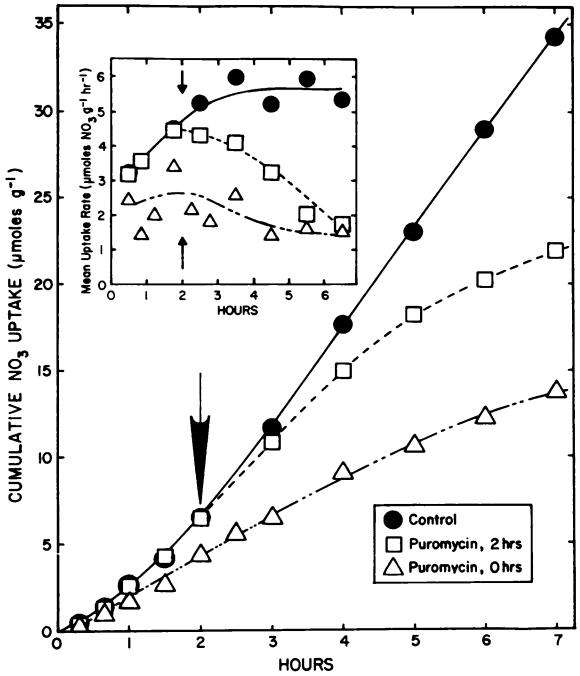


FIG. 13. Influence on nitrate uptake of puromycin (400 $\mu g$ $ml^{-1}$) applied at the outset and after 2-hr exposure to nitrate. Other conditions were as in Figure 1.

Table III. *Influence of Puromycin on Nitrate Uptake, Nitrate Translocation, and on Nitrate and Nitrite Reductase Activities of Corn Roots*

Values were obtained after 7-hr exposure to nitrate-containing solutions from plants whose nitrate uptake patterns are shown in Figure 13. Enzyme assays were made on apical 3.5-cm root segments. Values in parentheses are per cent of the treatment without puromycin.

| | Puromycin (400 $\mu g$ $ml^{-1}$) Treatment | | |
|---|---|---|---|
| | None | At start | After 2 hr |
| Nitrate uptake ($\mu$moles $g^{-1}$) | 34.7 | 12.6 (36) | 19.9 (57) |
| Nitrate in xylem exudate ($\mu$moles $g^{-1}$) | 4.34 | 1.25 (29) | 2.52 (58) |
| % Translocation | 12.5 | 9.9 (79) | 12.7 (102) |
| Nitrate reductase ($\mu$moles $g^{-1}$ $hr^{-1}$) | 0.59 | 0 (0) | 0.13 (22) |
| Nitrite reductase ($\mu$moles $g^{-1}$ $hr^{-1}$) | 16.1 | 9.7 (60) | 13.1 (82) |

nated at 3 C (Fig. 2) and was largely prevented when only excised roots were employed (Fig. 7). Translocation of nitrate to the xylem exudate also increased in rate after exposure of the roots to nitrate (Fig. 1) and was dependent upon continuous nitrate uptake; it declined sharply when exogenous nitrate was no longer available (Fig. 3). Despite the close association between uptake and translocation, the quantities of nitrate translocated to the xylem sap were not sufficient to account for the total increase in the nitrate uptake rate. It follows that an increase in uptake capacity must have occurred.

Nitrate was excluded (relative to chloride) from the root tissue during the lag period but during the accelerated phase the nitrate uptake rate exceeded the prior linear chloride uptake rate (Fig. 4). Similar differences in nitrate and chloride uptake patterns have been noted in 10-day-old nitrogen-

depleted wheat seedlings (11). These differences and the greater sensitivity of nitrate uptake to endosperm removal (Fig. 9) indicate that the processes involved in uptake of the two monovalent anions were not the same (2, 5, 29).

Development of the accelerated phase of nitrate uptake appeared to be specifically dependent upon prior uptake of nitrate. The seedlings had been exposed to 0.5 mM ammonium for 24 to 48 hr prior to the experiments and should have had adequate nitrogen for general metabolism. Pretreatments for 2 hr with 0.5 mM $NaNO_2$ did not shorten the lag period (Fig. 6). A relatively small amount of nitrate was sufficient to initiate the increase to the accelerated rate because an increase in rate could usually be detected by the 2nd hr, by which time less than 5 $\mu$moles nitrate $g^{-1}$ had entered the tissue.

**The Influence of the Endosperm.** When excised roots alone were used, nitrate uptake rates progressively declined after the 2nd hr (Fig. 7). This decline was delayed in seedlings whose endosperm had been removed (Fig. 8), but such seedlings did not develop the maximal rate observed in seedlings in which only the etiolated shoots had been removed (Fig. 8). Apparently, the endosperm (or scutellum to a lesser extent) supplied the roots with a substance (or substances) essential to full development of the accelerated phase. Respiratory measurements indicated a consumption of approximately 3.7 $\mu$moles glucose-equivalents $g^{-1}$ $hr^{-1}$. Assuming no input into the soluble sugar pool from outside the roots or from insoluble carbohydrates within the roots, about a third of the original soluble sugars (45 $\mu$moles $g^{-1}$) would have been consumed by 4 hr. At this time endosperm removal clearly had resulted in a lower nitrate rate (Fig. 8). It is also possible that a hormonal substance had to be translocated into the roots for maximal development of the accelerated phase. The consistently lower uptake rate during the accelerated phase in the intact seedlings, relative to seedlings whose shoots had been excised (Fig. 8), could have been caused by the etiolated shoots serving as a competitive sink for the required stimulatory substance(s).

**The Influence of RNA- and Protein-Synthesis Inhibitors.** The inhibitor studies suggest that RNA and protein synthesis were also required for full development of the accelerated phase of nitrate uptake. Cycloheximide, an inhibitor of protein synthesis, drastically inhibited nitrate uptake (Fig. 12). It has been noted (4) that cycloheximide depresses salt uptake, which would be expected if proteins are directly involved in the process. However, the inhibitor also affected energy transfer, which would result in restricted salt uptake.

Chloramphenicol at 50 $\mu$g $ml^{-1}$ had no significant influence on the nitrate uptake pattern (Fig. 12). At this concentration, chloramphenicol is relatively ineffective in inhibiting protein synthesis by the cytoplasmic ribosomal system in higher plants (3).

Puromycin (Fig. 13), which is presumed to compete with aminoacyl-tRNA during polypeptide synthesis thereby terminating protein synthesis (6, 31), restricted development of the accelerated rate (Fig. 13). Inhibition was evident within 1 hr when puromycin was applied after the increase in nitrate uptake rate had been initiated (Fig. 13). If in fact this effect of puromycin was a result of an influence on protein synthesis and not an indirect effect (*e.g.* on energy transfer), it would seem that continuous protein synthesis was required for development and maintenance of the maximal nitrate uptake rate during the accelerated phase. If so, a protein with exceptionally high turnover rate would be required.

There was a strong depressing effect on nitrate uptake by 0.5 mM 6-methylpurine (Fig. 10), a concentration reported to inhibit the synthesis of all forms of RNA (14). Actinomycin D, a specific inhibitor of DNA-dependent RNA synthesis (27, 28) also restricted nitrate uptake (Fig. 10 and 11). No consistent influence was observed with 5-flurouracil (Fig. 10) which (at 2.5 mM) inhibits synthesis of ribosomal RNA (14). Hence, the 6-methylpurine and actinomycin D effects apparently did not result from restricting the synthesis of this species of RNA. The failure of actinomycin D to have a significant effect until after 3 hr had passed (Fig. 11) suggests the possibility that a relatively long lived messenger RNA (16) was functional in promoting development of the accelerated rate of nitrate uptake, but slow penetration of actinomycin D into the tissue could explain the result equally well. The dangers in extrapolation of results of inhibitor studies with intact tissue have been consistently emphasized (4, 6, 15, 20). The implicated requirement for continuous protein synthesis is therefore tentative.

**The Induction of Nitrate Reductase.** A small quantity of nitrate was sufficient for the apparent induction of nitrate (and nitrite) reductase; an increase in enzyme activity was detectable after 1 hr and was clearly evident by the 2nd hr (Fig. 5). Nevertheless, continuous nitrate uptake was required to maintain the net synthesis of nitrate reductase in the apical 3.5-cm root segments. Failure of further increase in activity and its decline upon removal of ambient nitrate occurred in spite of the fact that the tissue contained 8.7 $\mu$moles nitrate $g^{-1}$ at the time of transfer (Fig. 5). Investigations with tobacco cell cultures also suggest that port of the incoming nitrate was secreted into storage pools which were not readily available for induction and maintenance of nitrate reductase activity (8). Nitrate in the storage pools of the roots also was effectively sequestered against translocation to the xylem (Fig. 3).

**The Relationship between Nitrate Reduction and the Onset of the Accelerated Phase of Nitrate Uptake.** Some of the data support the concept that these two processes were not directly linked. If events subsequent to the reduction of nitrate to nitrite (*e.g.*, an increased supply of reduced nitrogen) were required for development of the accelerated phase, pretreatments with nitrite should have hastened its appearance, but they failed to do so (Fig. 6). From measurements made at the end of the experimental period, 6-methylpurine (Table I, Fig. 10), actinomycin D (Tables I and II, Figs. 10 and 11), and puromycin (Table III, Fig. 13) all appeared to affect total nitrate uptake relatively less than synthesis of nitrate reductase. Nitrate reductase activity continued to increase for at least 6 hr (Fig. 5), whereas the nitrate uptake rate was relatively constant after the 3rd or 4th hr. Although not definitive, these observations support the hypothesis that separate events were involved in the apparent induction of nitrate uptake and the increase in nitrate reductase activity, and that continuous synthesis of a specific nitrate transport protein was required (25).

The alternative hypothesis, *i.e.*, that nitrate reductase induction was necessary for the accelerated nitrate uptake phase to develop has not been eliminated. Significant nitrate reduction could have occurred as soon as the accelerated uptake phase was evident (Figs. 1, 2, and 5). Nitrate reductase could itself act as a transport protein, a hypothesis which presumably would require that it occupy a membrane site. There is evidence for a particulate nitrate reductase in barley roots (1, 22), but recent studies by Dalling, Tolbert, and Hageman (unpublished data), suggest that the enzyme in corn roots is cytoplasmic. Indirect effects of the act of nitrate reduction may also be responsible for the increase in nitrate uptake. Cytoplasmic bicarbonate resulting from nitrate reduction (17) may serve to exchange with ambient nitrate (33). The end result would be similar if the increase in bicarbonate resulted in increased synthesis (12) and subsequent decarboxylation (30) of malate. It is also possible that nitrate reductase induction

and consequent nitrate reduction may increase glycolysis rates via NADH turnover (18), thereby increasing mitochondrial tricarboxylic acid cycle activity, electron transport, and energy production. But failure to detect consistent and significant effects of nitrate relative to chloride on $O_2$ uptake argues against this hypothesis.

Regardless of the regulatory factors involved, the present results show that apparent induction of nitrate uptake occurred with consistency, and that the endosperm supplied a substance(s) necessary for its maximal expression.

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