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Effects of Different Inorganic Nitrogen Sources on Photosynthetic Carbon Metabolism in Primary Leaves of Non-nodulated *Phaseolus vulgaris* L.[1]

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ABSTRACT

Young bean plants (*Phaseolus vulgaris* L. var Saxa) were fed with three different types of inorganic nitrogen, after being grown on nitrogen-free nutrient solution for 8 days. The pattern of $^{14}CO_2$ fixation was investigated in photosynthesizing primary leaf discs of 11-day-old plants (3 days with nitrogen source) and in a pulse-chase experiment in 13-day-old plants (5 days with nitrogen source).

Ammonium caused, in contrast to nitrate nutrition, a higher level of ^{14}C incorporation into sugar phosphates but a lower incorporation of label into malate, glycolate, glycerate, aspartate, and alanine. The labeling kinetics of glycine and serine were little changed by the nitrogen source. Ammonium feeding also produced an increase in the ratio of extractable activities of ribulose-1,5-bisphosphate carboxylase to phosphoenolpyruvate carboxylase and an increase in dark respiration and the CO_2 compensation concentration. Net photosynthesis was higher in plants assimilating nitrate.

The results point to stimulated turnover of the photosynthetic carbon reduction cycle metabolites, reduced phosphoenolpyruvate carboxylation, and altered turnover rates within the photosynthetic carbon oxidation cycle in ammonium-fed plants. Mechanisms of the regulation of primary carbon metabolism are proposed and discussed.

The effect of NH_4^+ on photosynthetic carbon assimilation has been repeatedly reported (3, 8, 12, 13, 16, 17, 19–23, 27, 29) and the changes in metabolism which occur in response to toxic NH_4^+ concentrations recently reviewed (10). Studies with *Chlorella* and *Medicago sativa* suggest that, during NH_4^+ assimilation, photosynthetically fixed carbon is diverted into amino acids at the expense of sucrose synthesis (12, 13, 22). Pyruvate kinase, PEP[2] carboxylase, and RuBP carboxylase are all apparently stimulated (8, 12, 13, 20–22), and in isolated spinach cells, photosynthetic $^{14}CO_2$ fixation increased, in response to the presence of NH_4^+ (29).

Supplying C_4 and C_3 plants with NH_4^+ as compared to NO_3^- produces, among other effects, changes in the initial carboxylation products, the CO_2 compensation point, and the net photosynthetic rate (26), while in maize, NH_4^+ nutrition causes a lower incorporation of label from $^{14}CO_2$ into malate (3). Further, the addition of NH_4^+ to NO_3^- grown cells of *Nicotiana tabacum* produced an increase in the cellular concentrations of alanine and glutamine, a decrease in the concentration of malate and an inhibition of malic enzyme activity (2). However, the supply of NO_3^- to cells of *Spinacia oleracea* had no apparent effect on the distribution of photosynthetic products compared with N-free nutrition, whereas in NH_4^+-fed cells, fixation of CO_2 into carbohydrates decreased but increased in carboxylic and amino acids (16).

The effect of inorganic N sources on the composition of N compounds in the bleeding sap and leaves of the bushbean *Phaseolus vulgaris* has previously been reported (6, 27). Coupled with the above observations, this has led us to investigate the effect of different N sources on various carboxylating enzymes and on the pattern of $^{14}CO_2$ fixation in primary leaves of non-nodulated bean plants grown in liquid culture. Our results show that different N sources, fed through the roots of *Phaseolus vulgaris*, cause changes in photosynthetic carbon metabolism.

MATERIALS AND METHODS

Plant Material. Seeds of *Phaseolus vulgaris L.* var Saxa (strain Vatter) were soaked overnight in tap water, rinsed, and placed on wet tissue paper in darkness for 3 d at 24 ± 2°C. Seedlings were transferred to coarse quartz sand, watered with N-free nutrient (27), and grown under an illumination of 7000 to 9000 lux. After a further 4 d (day 8), the plants were set into plastic tanks filled with 14 L nutrient solution containing a total N concentration of 3.5 mM either as NO_3^-, NH_4^+, or NH_4NO_3 (27). The pH of the nutrient solution was adjusted with either KOH or H_3PO_4. All subsequent investigations were carried out on primary leaves.

$^{14}CO_2/^{12}CO_2$ Pulse-Chase Experiments. After 5 days with the appropriate N supply (day 13) discs, 10 mm in diameter, were cut from the primary leaves. Thirteen discs were floated (adaxial side down) on 0.5 ml distilled H_2O in a 40-ml assimilation flask and flushed with 330 μl/l CO_2 in air at a rate of 20 l/h for 1 h prior to the pulse-chase experiment. Discs were then supplied with $^{14}CO_2$ for 60 s at a rate of 10 l/h followed by a chase period of 180 s with $^{12}CO_2$. The concentration of $^{14}CO_2$ was, in experiment A: 292 to 302 μl/l; B: 274 to 282 μl/l; and C: 258 to 268 μl/l. $^{14}CO_2$ was generated from 1 mCi $BaCO_3$ with phosphoric acid (specific activity, 10 mCi/mmol). The leaf discs were illuminated from below by fluorescent tubes (Philips TL 40W/33) with a light intensity of 10,000 lux (170 $\mu E/m^2 \cdot s$), and the flasks were incubated at 25°C in a water bath. Samples were inactivated 30, 60, 120, or 240 s after the beginning of the $^{14}CO_2$ pulse by injection of cooled (−60°C) absolute ethanol into the flasks.

In addition to the pulse-chase experiment, the incorporation of $^{14}CO_2$ into primary leaves of 11-d-old bean plants was followed with labeling times of 7, 15, 30, and 60 s. The CO_2 concentration in this experiment was 240 to 260 μl/l and the flow rate 25 l/h. Here, plants had previously been fed with either NO_3^-, NH_4^+, or NH_4NO_3, or, in addition, with a N-free nutrient solution.

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[2] Abbreviations: RuBP, ribulose 1,5-bisphosphate; PEP, phosphoenolpyruvate; PCR, photosynthetic carbon reduction; PCO, photosynthetic carbon oxidation; PGA, phosphoglycerate.

Extraction and Separation of ^{14}C-Labeled Products. Leaf discs were extracted in 80 and 20% ethanol for 20 min and 15 min, respectively, at 60°C. The remaining residue was homogenized in distilled H_2O and the radioactivity in the insoluble fraction was determined.

After rotary evaporation and resuspension in alcohol:water (1:2, v/v), chloroform was added and mixed thoroughly, and apolar substances were centrifuged into the chloroform phase and rejected. The alcohol-soluble fraction was passed through a Pasteur pipette containing Dowex 50Wx8 (200/400 mesh, hydrogen form) and the column was washed several times with distilled H_2O to elute the neutral fraction containing organic acids, sugar phosphates, and free sugars. Cationic compounds (mostly amino acids) were eluted from the column with 10% (v/v) NH_4OH. The cationic and neutral fractions were rotary-evaporated to dryness at 40°C and redissolved in distilled H_2O. Products were then separated by two-dimensional TLC. Components of the neutral fraction were separated by developing the plates first in EDTA:NH_4OH (25% in water):*n*-propanol:isopropanol:*n*-butanol:isobutyric acid:water (0.24:20:70:15:15:500:190, w/v/v/v/v/v/v) for 24 h with overrun tapes, and then at 90° to the first direction in 2-methyl-2-butanol:*p*-toluol sulfonic acid:water (30:1:15, v/w/v) for 16 h. Components of the cationic fraction were separated on thin layer plates developed with *n*-butanol:acetone:NH_4OH (25% in water):water (10:10:2:5, v/v/v/v) for 3 h followed by 6 h at 90° to the first direction in isopropanol:formic acid:water (20:1:5, v/v/v). Labeled compounds were located by autoradiography, removed from the plates, and counted on a Picker Nuclear Liquimat 220 scintillation counter.

Extraction and Assay of Enzymes. Bean leaves were homogenized in 3 volumes of 0.1 M Tris-HCl, pH 8.0, containing 10 mM EDTA-Na_2, 20 mM $MgCl_2$, 5 mM DTT, and 1% (w/v) insoluble PVP, using a Polytron homogenizer. After filtration through one layer of Miracloth, the filtrate was centrifuged at 25,000*g* for 10 min at 4°C. One ml clear supernatant was loaded onto a Sephadex G-25 column (bed volume, 5 ml; 11 × 62 mm), previously equilibrated with 0.1 M Tris-HCl (pH 8.5) containing 20 mM $MgCl_2$, 10 mM $NaHCO_3$, and 1 mM DTT, and centrifuged at 300*g* for 1 min, then 1,000*g* for 1 min in order to eliminate excess buffer. After 10 min, the Sephadex column was centrifuged again, as described above, and the eluant was used for determining enzyme activities.

PEP carboxylase (EC 4.1.1.31) was assayed by the method of Lane *et al.* (15) in polystyrole microtubes sealed with serum caps. The reaction mixture contained 50 μmol Tris-HCl, pH 8.5, 5 μmol $MgCl_2$, 2.5 μmol reduced glutathione, 15 units malate dehydrogenase, 1 μmol PEP in a final volume of 0.5 ml and was flushed with N_2 for 1 min. The reaction was initiated after adding 10 μmol $NaH^{14}CO_3$ (50 nCi/μmol) by the injection of 50 μl extract. After 10-min incubation at 25°C, the reaction was stopped with 100 μl 2 N HCl. 100 μl of sample was dried at 90°C for 30 min, and then cooled, and 10 ml scintillation fluid (12 g PPO, 0.36 g POPOP, 2L toluol, 1 L Triton X-100) were added. Radioactivity was determined as before. RuBP carboxylase (EC 4.1.1.39) was assayed according to Lorimer *et al.* (18) in the same manner as PEP carboxylase.

CO_2 Gas Exchange Measurements. Four to 12 whole primary leaves were removed under water and placed in a Plexiglass chamber (volume, 100 cm^3) with the leaf stems immersed in distilled H_2O. CO_2 was monitored with a URAS I (Hartmann and Braun, Stuttgart) IR gas analyzer. The flow rate of the air stream was 36 l/h (21% O_2, open system: 330 μl/l CO_2) and the temperature in the chamber was 25°C under Iod quartz lamps (Philips 12013 R/1000W) giving a light intensity of 11,000 lux on the leaf surfaces.

RESULTS

CO_2 Gas Exchange Measurements. Net photosynthesis in the primary leaves of beans decreased between days 3 to 7 after transition to NO_3^- as sole N source from 175 to 150 μmol CO_2/dm^2 · h (data not shown). In comparison, the photosynthetic rates of NH_4-fed plants decreased during the same period from 160 to 100 μmol $CO_2/dm^2 \cdot h$, while their CO_2 compensation concentration and dark respiration increased (Fig. 1). Beans fed NH_4NO_3 had photosynthetic and respiratory rates similar to NO_3^--fed plants.

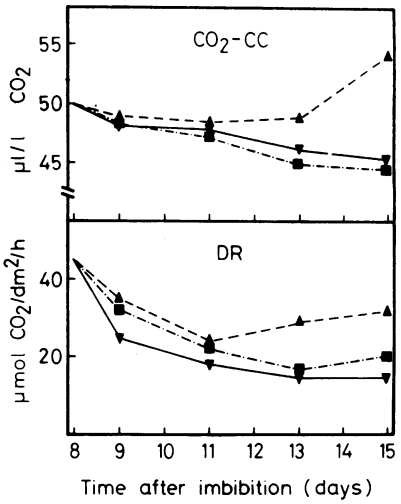

FIG. 1. CO_2 compensation concentration (CO_2-CC) and dark respiration (DR) rates from detached primary leaves of bean plants, which received NO_3^- (▼——▼), NH_4^+ (▲- - -▲), or NH_4NO_3 (■-·-■) as N source 8 d after imbibition.

Photosynthetic $^{14}CO_2$ Incorporation. When primary leaf discs of 11-d-old plants (3 d on nutrient solution) were allowed to fix $^{14}CO_2$, the total incorporation of label into the tissue was apparently not influenced by the N source. Discs of 13-d-old plants (5 d on nutrient solution), however, fixed different amounts of $^{14}CO_2$, depending on their N source (Fig. 2). In agreement with the results obtained from gas exchange measurements, plants grown on NH_4^+ only showed the lowest ^{14}C incorporation. There was no remarkable difference in the distribution of label between the cationic and neutral fractions, in either 11- or 13-d-old plants, with the exception of leaves pretreated with a N-free nutrient where a relative increase in activity in the neutral fraction was observed (Fig. 3).

The distribution of labeled carbon among metabolites after two-dimensional thin layer separation of both fractions demonstrated a marked effect of the N source on leaf metabolism. A notably higher incorporation of label during the pulse, followed by a more rapid loss of label during the chase period, was found in PCR cycle intermediates (PGA, see Fig. 4; data for RuBP and ribose-5-P not shown) and PEP (see Fig. 5) of NH_4^+-fed plants than of either NO_3^-- or NH_4NO_3-treated beans. The leaves of N-starved plants of 11-d-old beans showed a similar labeling pattern to those supplied with NH_4^+. A more rapid incorporation of label into glucose-P was observed in leaves of NH_4^+-fed beans, but the activity declined slowly during the chase period (Fig. 4).

The label of malate, aspartate, and alanine (Fig. 5) was higher in the leaf discs of NO_3^-- and NH_4NO_3-treated beans throughout the pulse-chase period, compared with much lower levels of labeling in NH_4^+-fed plants. Very similar results were obtained with 11-d-old beans (Fig. 3, C and D) with N-starved plants again mimicking NH_4^+-fed beans in the labeling kinetics of malate and aspartate.

The various N sources also apparently influenced the distribution of label between photorespiratory intermediates. Incorporation of ^{14}C into glycine and serine was largely unaffected by the various N sources. The labeling of serine increased under all nutrient conditions during the chase period. The different N

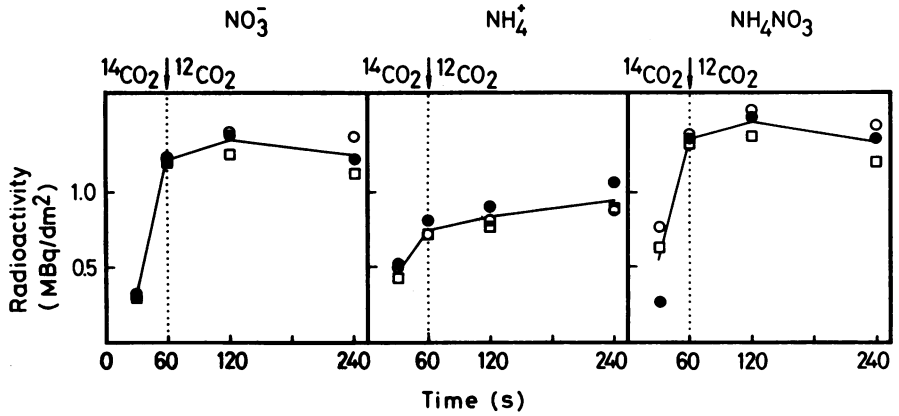

FIG. 2. Effect of NO_3^-, NH_4^+, and NH_4NO_3 nutrition on total photosynthetically incorporated ^{14}C by primary leaf discs of 13-d-old bean plants in a $^{14}CO_2/^{12}CO_2$ pulse-chase experiment (5 d with 3.5 mmol N/L nutrient solution). The discs were exposed during the pulse to a $^{14}CO_2$ concentration (specific activity, 10 mCi/mmol) in air of 292 to 302 μl/l (○), 274 to 282 μl/l (●), and 258 to 268 μl/l (□).

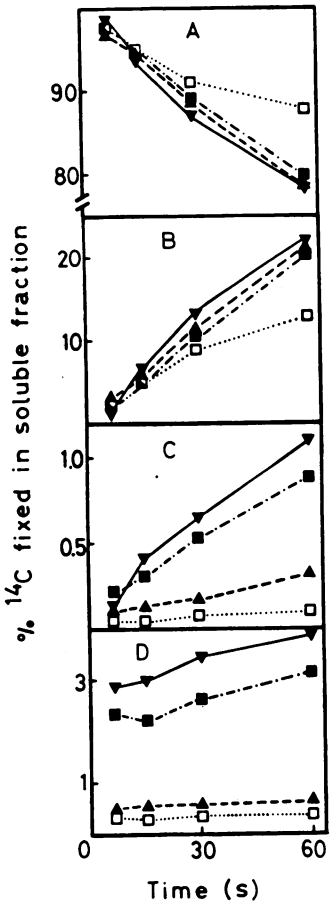

FIG. 3. Effect of different conditions of N nutrition on the ^{14}C incorporation in the neutral fraction (A), the cationic fraction (B), aspartate (C), and malate (D) as a percentage of the total activity recovered from the soluble fraction of $^{14}CO_2$ photosynthesizing primary leaf discs of 11-d-old bean plants after 3 d on the different nutrient solutions. The nutrient solutions contained as N source (3.5 mmol N/L) NO_3^- (▼——▼), NH_4^+ (▲- - -▲), or NH_4NO_3 (■-·-■) and no N (□···□). The CO_2 concentration (specific activity, 10 mCi/mmol) in air was 240 to 260 μl/l.

regimes had a more marked effect on the labeling patterns of glycolate and glycerate (Fig. 6). Ammonium supply led to a lower ^{14}C incorporation into both.

Enzyme Activities. The extractable activities of RuBP carboxylase and PEP carboxylase were measured in the primary leaves of beans supplied with various nitrogenous sources over a period of 9 d (Fig. 7). Ammonium nutrition caused a sharp increase of RuBP carboxylase activity during the first 4 d, followed by a rapid decline. No significant increase was induced by NO_3^- and only a slight increase by NH_4NO_3. PEP carboxylase activity was higher (up to 70%) in NO_3^--fed bean leaves than in NH_4^+-fed plants. Consequently, the ratio of RuBP carboxylase to PEP carboxylase increased in NH_4^+-fed plants to a maximum after 4 d and then declined. For NO_3^-- and NH_4NO_3-treated beans, the ratio increased steadily but more slowly throughout the 9 d on nutrient solution.

DISCUSSION

Abscission of primary leaves occurred after 9 to 10 d of growth on 3.5 mM NH_4^+ without the leaves first turning yellow and after diurnal movement had ceased. This premature senescence must be attributed to an induced metabolic insufficiency rather than to the normal process of senescence. The visible effects of NH_4^+ nutrition seems to be restricted to the primary leaves because the development of the trifolia shows no impairment. During the first 3 d of growth on different N sources, net photosynthesis remained unchanged (data not shown) but thereafter a reduction in the net rate of $^{14}CO_2$ fixation occurred in leaves of NH_4^+-grown beans by as much as 30 to 45% of that of NO_3^--fed plants (Fig. 2). A reduced PEP carboxylation (as discussed later) and an enhanced rate of dark respiration (if it persists in the light)(Fig. 1) might reduce net $^{14}CO_2$ assimilation. The disequilibration of the carboxylating and decarboxylating reactions is reflected in the rapid increase in the CO_2 compensation concentration (Fig. 1) 5 d after the beginning of NH_4^+ nutrition.

An interpretation of the pattern of ^{14}C fixation in the pulse-chase experiments has to take into consideration that no N compounds or other mineral nutrients were supplied to the leaf discs during the experiments, including 1 h of adaptation to conditions prior to the pulse-chase. Therefore, there could not have been a direct influence by exogenous N compounds. It is suggested that the leaf discs used in these experiments were representative of primary leaves adapted to the nutritional conditions established by the composition, concentration (27), and flow rate of the xylem sap.

It has been repeatedly shown that NH_4^+-fed plants incorporate a greater amount of photosynthetically fixed ^{14}C into the total amino acid pool, at the expense of carbohydrate synthesis, compared with NO_3^--fed or N-starved plants (2, 21–23). In bean leaf discs, no marked differences were found between plants adapted to NO_3^-, NH_4^+, or NH_4NO_3 regarding the amount of label incorporated in the soluble and insoluble fractions (data not shown) or

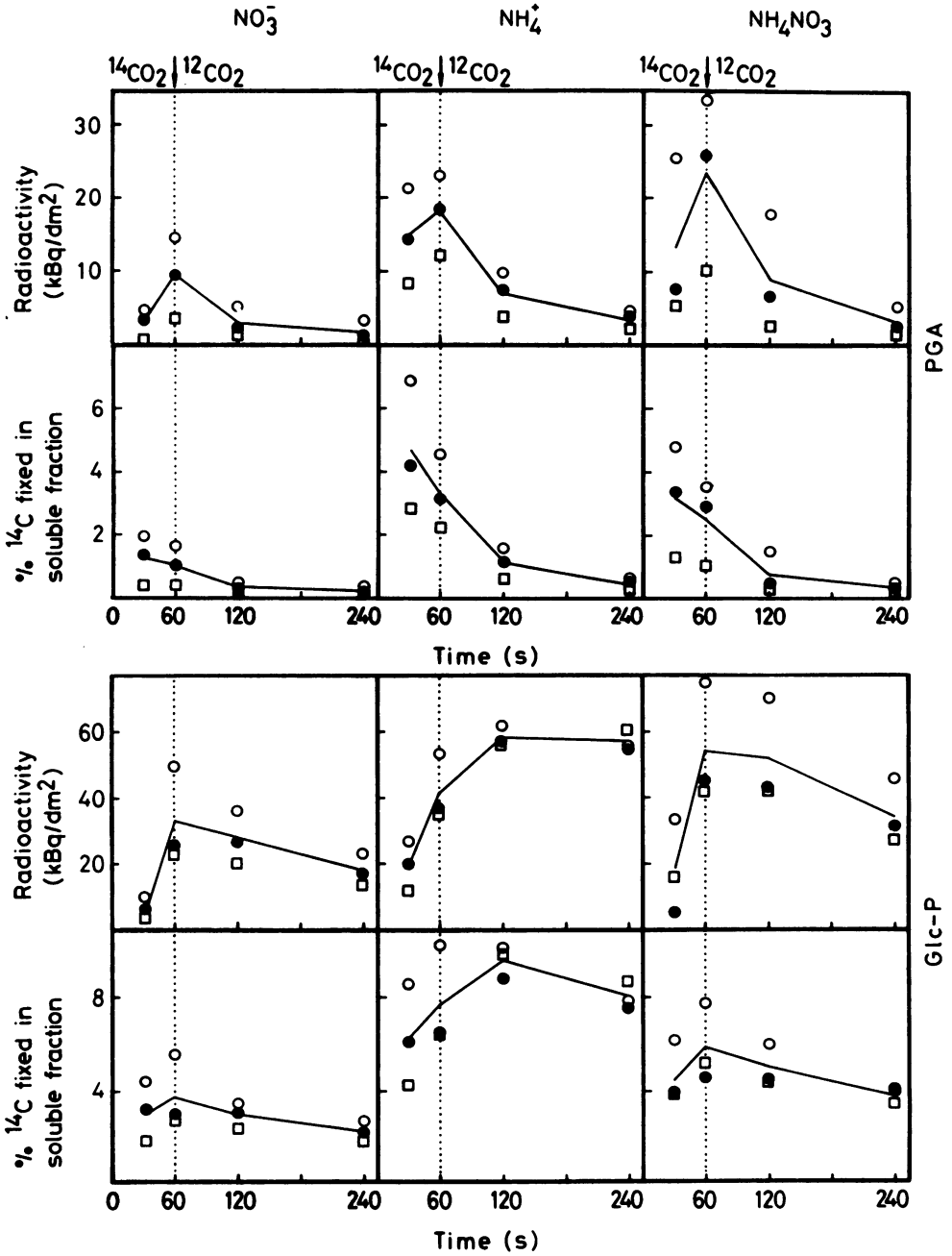

FIG. 4. Radioactivity of glucose monophosphate (Glc-P) and PGA is expressed as kBq/dm^2 leaf area and as a percentage of the total activity recovered from the soluble fraction of the exposed primary leaf discs (conditions as in Fig. 2).

in the total pools of amino acids (cationic fraction) and of N-free compounds (neutral fraction) (Fig. 3). As expected, in N-starved plants, a higher proportion of label remained in the neutral fraction. The implication of the results is that in N-starved plants incoporation of label into amino acids is due to transamination processes in an actively turning over pool of amino acids, and that in N-fed plants the actively turning over pool is much larger.

Photosynthetic Carbon Reduction Cycle. The pattern of incorporation of $^{14}CO_2$ into PGA (Fig. 4) and the increased extractable activity of RuBP carboxylase in NH_4^+-fed beans (Fig. 7) compared to NO_3^--grown plants indicate the stimulation of RuBP carboxylation by NH_4^+. A similar observation was made in experiments with *Dunaliella tertiolecta* (20) and spinach chloroplasts (8). The levels of ^{14}C remaining in glucose-P (Fig. 4) during the chase period of NH_4^+-fed plants suggest that the synthesis of carbohydrates through glucose-P is reduced, in agreement with previously published results (3, 13).

In primary leaves of NO_3^--fed beans, a high level of unbound NO_3^- is present (27). Reduction of NO_3^- to NH_4^+ consumes reducing equivalents and the ratio NADPH/NADP$^+$ in leaves of NH_4^+-fed plants has been shown to be higher than in plants supplied with NO_3^- (28). NADPH, but not NADP$^+$, apparently stimulates RuBP carboxylase activity (5) and it may be that the turnover of carbon through the PCR cycle is partly regulated by the N source through the relative levels of oxidized and reduced nucleotides.

PEP Carboxylation. The labeling of malate, aspartate, and PEP (Figs. 3 and 5) and the lower extractable activity of PEP carboxylase (Fig. 7) point to a reduced level of PEP carboxylation in NH_4^+-grown beans. The ratio of the activities of RuBP carboxylase to PEP carboxylase (Fig. 7) may indicate that the PEP carboxylation plays a more significant role in NO_3^--fed beans. The change

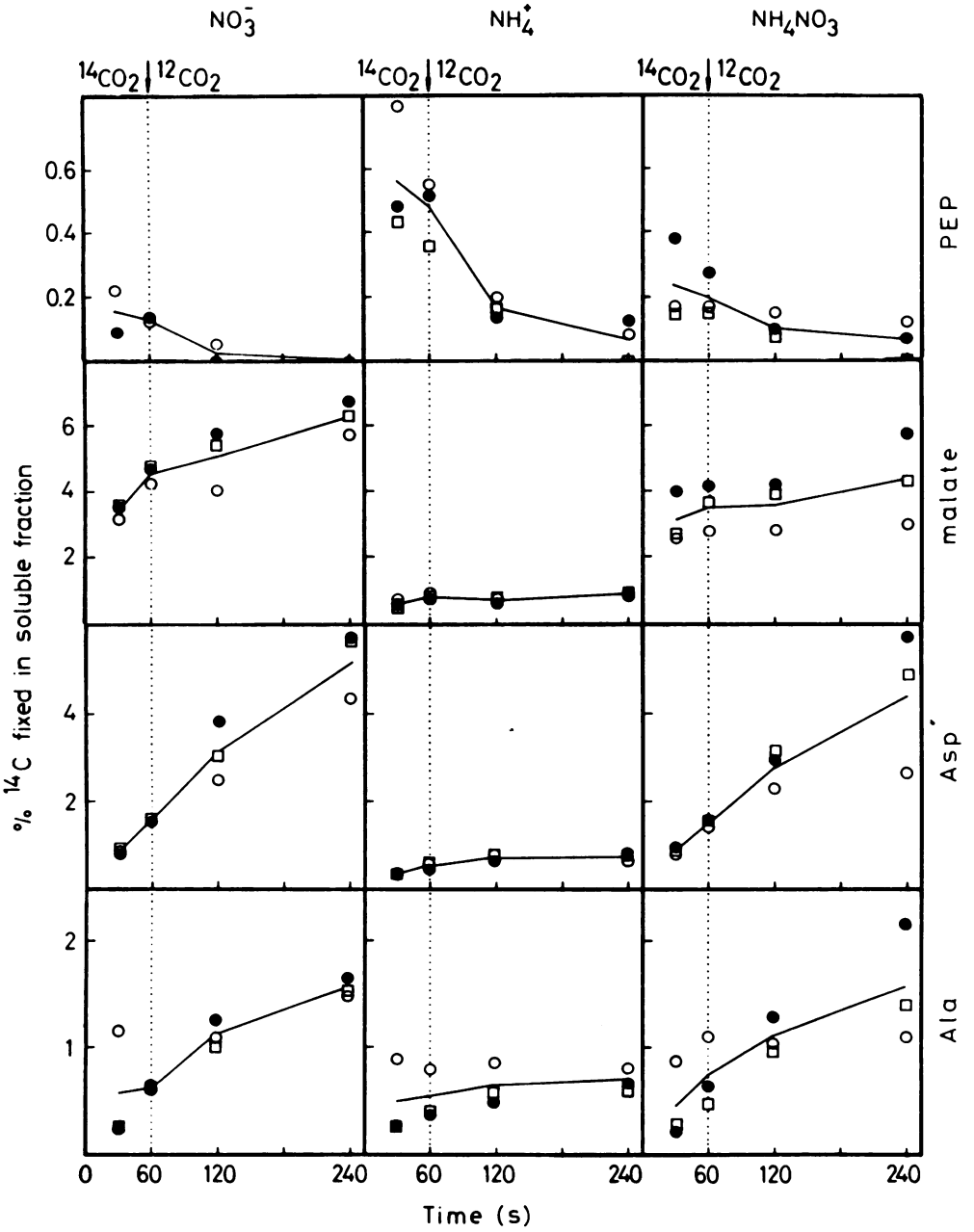

FIG. 5. ^{14}C incorporated in PEP, malate, aspartate, and alanine as a percentage of the total activity recovered from the soluble fraction of the exposed bean primary leaf discs (conditions as in Fig. 2).

in C-flux into malate could originate from the inhibition of malic enzyme as reported in NO_3^--grown tobacco cells after addition of NH_4^+ (2). This interpretation presumes an inhibition of PEP carboxylase by malate, perhaps in combination with a lower pH (7). Inhibition of malic enzyme activity may also account for the lower incorporation of ^{14}C into alanine by NH_4^+-fed beans (Fig. 5). Because malate is an important metabolite in NO_3^- transport and assimilation (1, 30), it may be that in NO_3^--fed beans there is an increase in malate synthesis as suggested by the results and as has been demonstrated in maize (3).

Photosynthetic Carbon Oxidation Cycle. The rapid turnover of label in glycerate (Fig. 6) during the chase period and the results of serine (data not shown) are in keeping with the view that the formation of glycerate takes place through P-glycerate and not through serine. Compared with NO_3^- treatment, NH_4^+-fed plants showed a reduced incorporation of label into glycerate but an increase in P-glycerate (Fig. 4), implying that in this situation the activity of P-glycerate phosphatase is inhibited, thereby maintaining a higher level of P-glycerate in the Calvin cycle. This would, in part, explain the increased level of labeling of PCR intermediates. An inhibition of the glycerate pathway in NH_4^+-fed bean leaves should lead to a lower incorporation of ^{14}C into serine, but this, however, is not the case. The pattern of labeling in serine and glycine throughout the pulse-chase are not influenced to the same extent by the N source, as for glycolate and glycerate (Fig. 6). Hydroxypyruvate was found to inhibit glutamate:glyoxylate aminotransferase (D. M. Luescher and K. H. Erismann, unpublished). It is possible, therefore, that the glycolate pathway is regulated by a metabolite of the glycerate pathway. The course of ^{14}C-labeling of glycine (data not shown) and glycolate (Fig. 7) points to a stimulation of the glycolate pathway when NH_4^+ is the N source. The more active pool of RuBP probably results in a higher RuBP oxygenase activity, borne out by the fact that the extractable activity of the enzyme is indeed, at first, higher in NH_4^+-fed plants

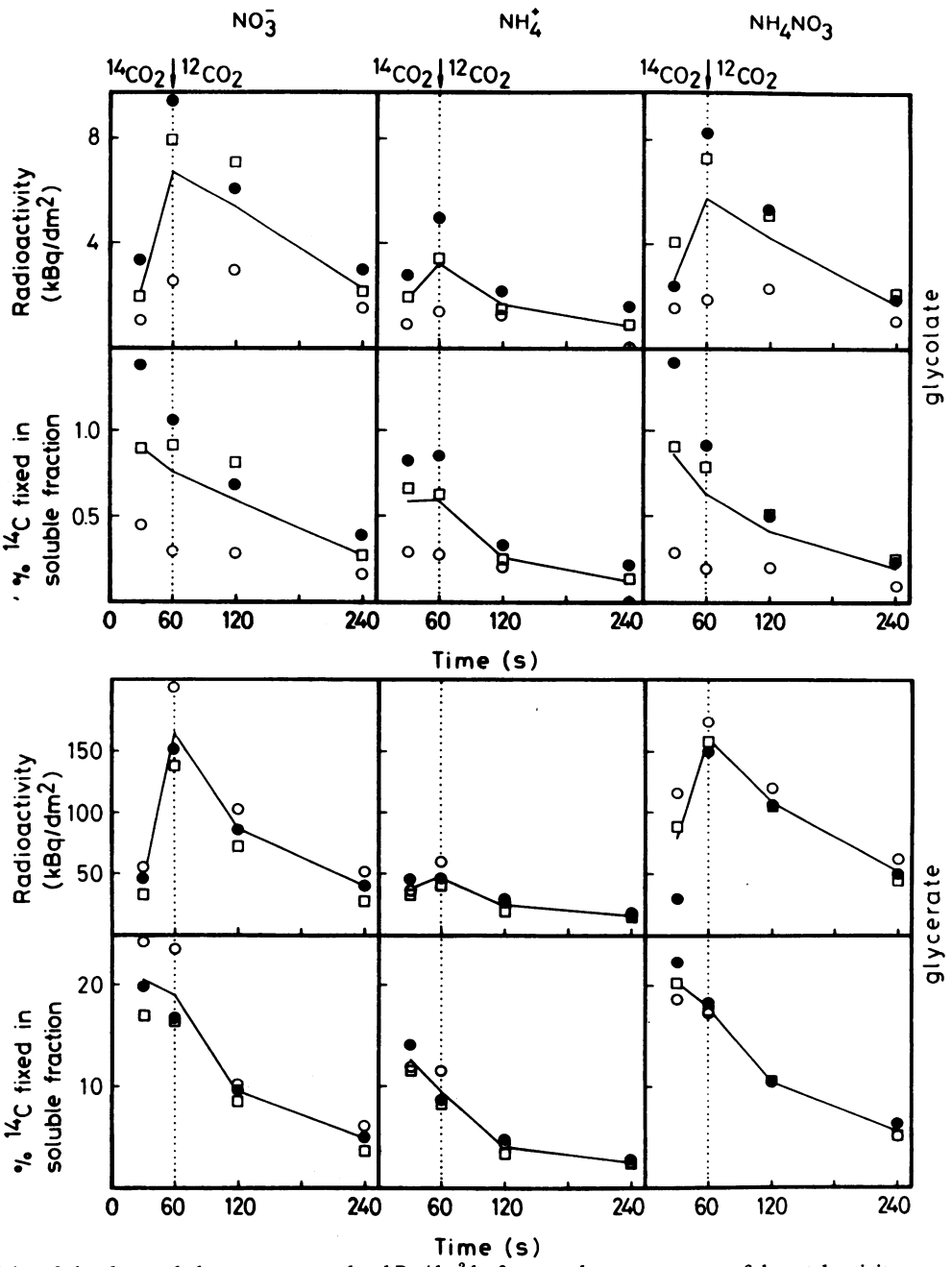

FIG. 6. Radioactivity of glycolate and glycerate expressed as kBq/dm^2 leaf area and as a percentage of the total activity recovered from the soluble fraction of the exposed bean primary leaf discs (conditions as in Fig. 2).

although the ratio of carboxylase/oxygenase is unchanged by the N source (data not shown). In keeping with this is recent evidence that the activity of glycolate oxidase of *Lemna minor* L. is increased by NH_4^+ nutrition (9). Thus, we suggest that the maintenance of the pool sizes of glycine and, especially serine are guaranteed during NO_3^- assimilation by the glycerate pathway, and during growth on NH_4^+, by the glycolate pathway.

Feeding of N through the roots raises the question of whether NH_4^+ or NO_3^- ions affect carbon metabolism directly or indirectly. In roots of *Phaseolus* and maize, NH_4^+ is transported mainly as allantoic acid, amides, and amino acids into the shoot, and only traces of free NH_4^+ ions are detectable (11, 27). The NH_4^+ concentration in the primary leaves of NH_4^+-or NO_3^--fed *Phaseolus* is low and is not altered significantly by the N source (data not shown), suggesting that the effect of NH_4^+ is indirect. The ^{14}C-labeling characteristics of N-free and NH_4^+-grown beans are similar, and contrast with the similar results obtained after growth on NO_3^- or NH_4NO_3. Moreover, the NO_3^- content in the xylem sap and in the primary leaves of NO_3^-- and NH_4^+-fed plants is completely different (27), inviting consideration of the NO_3^- ion as an immediate effector of carbon metabolism. Indirect effects of NH_4^+ must be considered in addition to effects produced by the absence of the NO_3^- ion, because primary leaves of NH_4^+-supplied beans senesce prematurely. One possibility is the acidification of the cell. The pH of the homogenate from primary leaves (in 3 volumes of water) was lowered during NH_4^+ feeding and N starvation to a value between 5.6 to 5.8. In beans grown on other forms of N, the pH decreased only to a value of 6.0 to 6.1. It is worth noting that a number of investigations have shown that the PCR and PCO cycle, and the relative activities of the C_4 and C_3 pathways are influenced by pH (4, 14, 24, 25). Decrease in pH produces effects similar to those observed in NH_4^+-fed beans but

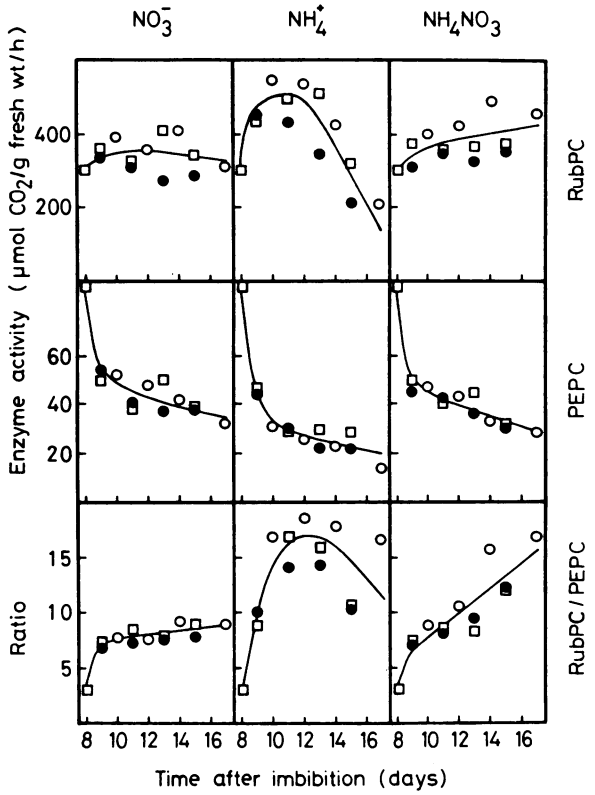

FIG. 7. Effect of NO_3^-, NH_4^+, and NH_4NO_3 nutrition on the extractable activities of ribulose 1,5-bisphosphate (RuBPC) and phosphoenolpyruvate carboxylase (PEPC) in bean primary leaves. The values are derived from three independent experiments (○, □, ●).

because we can neither measure the pH of cellular compartments nor hold pH constant within the cell, we are unable at present to ascertain to what extent the NH_4^+ nutrition-stimulated effects are overlaid by a pH effect.

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LITERATURE CITED

1. BEN ZIONI A, Y VAADIA, SH LIPS 1971 Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiol Plant 24: 288–290
2. BERGMANN L, W. GROSSE, P. KOTH 1976 Influences of ammonium and nitrate on N-metabolism, malate accumulation and malic enzyme activity in suspension cultures of *Nicotiana tabacum* var. "SAMSUN". Z Pflanzenphysiol 80: 60–70
3. BLACKWOOD GC, BJ MIFLIN 1976 The effect of nitrate and ammonium feeding on carbon dioxide assimilation in maize. J Exp Bot 27: 735–747
4. BOECHER M, M KLUGE 1978 The C_4-pathway of C-fixation in *Spinacea oleracea*. I. ^{14}C-labelling patterns of suspended leaf slices as influenced by the external medium. Z Pflanzenphysiol 83: 347–361 II. Pulse chase experiments with suspended leaf slices. Z Pflanzenphysiol 86: 405–421
5. CHU DK, JA BASSHAM 1974 Activation of ribulose 1,5-diphosphate carboxylase by nicotinamide adenine dinucleotide phosphate and other chloroplast metabolites. Plant Physiol 54: 556–559
6. COOKSON C, H HUGHES, J COOMBS 1980 Effects of combined nitrogen on anaplerotic carbon assimilation and bleeding sap composition in *Phaseolus vulgaris* L. Planta 148: 338–345
7. DAVIS DD 1979 The central role of phosphoenolpyruvate in plant metabolism. Annu Rev Plant Physiol 30: 131–158
8. DEBENEDETTI E, G FORTI, FM GARLASCHI, L ROSA 1976 On the mechanism of ammonium stimulation of photosynthesis in isolated chloroplasts. Plant Sci Lett 7: 85–90
9. EMES MJ, KH ERISMANN 1982 The influence of the nitrogen supply on the structure and activity of glycolate oxidase in *Lemna minor* L. Plant Sci Lett 27: 103–109
10. GIVAN CV 1979 Metabolic detoxification of ammonium in tissues of higher plants. Phytochemistry 18: 375–382
11. IVANKO S, J INGVERSEN 1971 Investigation on the assimilation of nitrogen by maize roots and the transport of some major nitrogen compounds by xylem sap. III. Transport of nitrogen compounds by xylem sap. Physiol Plant 24: 355–362
12. KANAZAWA T, K KANAZAWA, MR KIRK, JA BASSHAM 1972 Regulatory effects of ammonia on carbon metabolism in *Chlorella pyrenoidosa* during photosynthesis and respiration. Biochim Biophys Acta 56: 656–669
13. KANAZAWA T, MR KIRK, JA BASSHAM 1970 Regulatory effects of ammonia on carbon metabolism in photosynthesizing *Chlorella pyrenoidosa*. Biochim Biophys Acta 205: 401–408
14. KRAPF G, G JACOBI 1975 The pH dependence of CO_2 fixation and carbon assimilation in isolated spinach chloroplasts. Plant Sci Lett 5: 67–71
15. LANE MD, H MARUYAMA, RL EASTERDAY 1969 Phosphoenolpyruvate carboxylase from peanut cotyledons. Methods Enzymol 13: 277–283
16. LARSEN PO, KL CORNWELL, SL GEE, JA BASSHAM 1981 Amino acid synthesis in photosynthesizing spinach cells. Plant Physiol 68: 292–299
17. LAWYER AL, KL CORNWELL, PO LARSEN, JA BASSHAM 1981 Effects of carbon dioxide and oxygen on the regulation of photosynthetic carbon metabolism by ammonia in spinach mesophyll cells. Plant Physiol 68: 1231–1236
18. LORIMER GH, MR BADGER, TJ ANDREWS 1977 D-Ribulose-1,5-bisphosphate carboxylase-oxygenase. Improved methods for the activation and assay of catalytic activities. Anal Biochem 76: 66–75
19. MOHAMED AH, A GNANAM 1979 A possible mechanism of ammonium ion regulation of photosynthetic carbon flow in higher plants. Plant Physiol 64: 263–268
20. PAASCHE E 1971 Effect of ammonia and nitrate on growth, photosynthesis and ribulosediphosphate carboxylase content of *Dunaliella tertiolecta*. Physiol Plant 25: 294–299
21. PAUL JS, KL CORNWELL, JA BASSHAM 1978 Effects of ammonia on carbon metabolism in photosynthesizing isolated mesophyll cells from *Papaver somniferum* L.. Planta 142: 49–54
22. PLATT SG, Z PLAUT, JA BASSHAM 1977 Ammonia regulation of carbon metabolism in photosynthesizing leaf discs. Plant Physiol 60: 739–742
23. REHFELD DW, RG JENSEN 1973 Metabolism of separated leaf cells. III. Effects of calcium and ammonium on product distribution during photosynthesis with cotton cells. Plant Physiol 52: 17–22
24. ROBINSON JM, M GIBBS, DN COTLER 1977 Influence of pH upon the Warburg effect in isolated intact spinach chloroplasts. Plant Physiol 59: 530–534
25. SERVAITES JC, WL OGREN 1977 pH dependence of photosynthesis and photorespiration in soybean leaf cells. Plant Physiol 60: 693–696
26. TEW J, CF CRESSWELL, P FAIR 1975 The effect of nitrate and ammonia on the initial carboxylation product and the abundance of microbodies in some C_4 plants and *Hordeum vulgare* L. *In* M Avron, ed, Proceedings of the Third International Congress on Photosynthesis, vol 2. Elsevier, Amsterdam, pp 1249–1266
27. THOMAS RJ, U FELLER, KH ERISMANN 1979 The effect of different inorganic nitrogen sources and plant age on the composition of bleeding sap of *Phaseolus vulgaris* L.. New Phytol 82: 657–669
28. WEISSMANN GS 1972 Influence of ammonium and nitrate nutrition on the pyridine and adenine nucleotides of soybean and sunflower. Plant Physiol 49: 142–145
29. WOO KC, DT CANVIN 1980 Effect of ammonia on photosynthetic carbon fixation in isolated spinach leaf cells. Can J Bot 58: 505–510
30. WOO KS, M JOKINEN, DT CANVIN 1980 Reduction of nitrate via a dicarboxylate shuttle in a reconstituted system of supernatant and mitochondria from spinach leaves. Plant Physiol 65: 433–436