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Effect of Nitrate in the Rooting Medium on Carbohydrate Composition of Soybean Nodules[1]

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ABSTRACT

Nodulated soybean plants (*Glycine max* [L.] Merr) were grown in sand culture. Carbohydrate composition of nodules, roots, and leaf blades was determined and related to the effects of nitrate in nutrient solution on nodule growth and on nitrogenase activity of nodules.

When plants were grown without nitrate for 6 weeks and then supplied with 150 milligrams NO_3-N/liter for 4 or 7 days, there was an 80% decline in acetylene reduction activity of nodulated roots, relative to the 0-N control. The 80% decline in nitrogenase activity was accompanied by a decline in nodule glucose concentration of about 0.15 milligram per gram fresh weight and an increase in nodule sucrose concentration of about 0.23 milligram per gram fresh weight.

Plants were grown with 0, 30, or 100 milligrams NO_3-N/liter for 5 or 6 weeks to study long-term effects of nitrate on nodule growth. The 100-N treatment reduced nodule weight/plant by 70% but reduced the sum of sucrose + glucose + fructose concentration in nodules by only 12%. The ratios of [sucrose] in nodules/[sucrose] in roots and [fructose] in nodules/[fructose] in roots increased slightly in response to nitrate, indicating that nitrate affects sugar concentration in roots more than sugar concentration in nodules.

The effect of nitrate on glucose concentration of nodules was consistently negative. However, if it is assumed that sucrose, glucose, and fructose are equivalent in their ability to support nodule functions, then the overall results are not consistent with the hypothesis that nitrate inhibits nodule growth and activity by reducing the accumulation of carbohydrate in nodules.

The depressive effect of nitrate on root nodule growth has been known for more than a century. Reports on this subject published prior to 1900 were summarized by Fred and Graul in 1916 (4).

In 1920, Strowd reported that the presence of nitrate in the rooting medium lowered the concentration of reducing sugars in sap collected by maceration of soybean shoots or roots (24). Orcutt and Wilson (12) extended this observation by using more nitrate concentrations and by division of shoots into leaves and stems, and they concluded that "the effect of combined nitrogen on nodulation is probably an indirect one, *viz.*, the presence of combined nitrogen lowers the sugar content, which affects the development of nodules". This conclusion was supported by evidence that the negative effect of nitrate on number and weight of clover nodules could be partially overcome by increasing CO_2 concentration supplied to the shoots (5).

The concept that the negative effect of nitrate on nodule development is mediated via a reduction in carbohydrate accumulation in nodules has recently been rediscovered (6, 11, 17, 18). Most of the recent support for the concept has been derived from demonstrations that the proportion of $^{14}CO_2$ assimilated by shoots which is transported to nodules is reduced when plant roots are supplied with nitrate (10, 14, 18). Wong has shown recently that supplying sugars with nitrate to nodulated lentil roots nullifies the negative effect of nitrate on specific acetylene reduction activity of nodules (25). No evidence was presented to show that carbohydrate actually entered roots or nodules but results did show that carbohydrates significantly reduce nitrate uptake (25). Thus, Wong's results do not provide clear support for the idea that carbohydrate concentration in nodules limits nitrogenase activity in the presence of nitrate.

Previous workers have not assessed the effects of nitrate on the carbohydrate status of legume nodules by direct measurement of the carbohydrate composition of the nodules. Results reported here show little effect of nitrate on carbohydrate composition of soybean nodules when plants were grown in full sunlight with nitrate concentration in nutrient solution high enough to reduce nodule mass/plant by about 70%. Results also show that large reductions in nitrogenase activity caused by supplying plants with nitrate were accompanied by only small changes in carbohydrate compositions of nodules.

MATERIALS AND METHODS

Plant Culture. Soybean seeds (*Glycine max* [L.] Merr.), cv. "Beeson" were planted in silica sand and inoculated with a commercial preparation of *Rhizobium japonicum* (20). All nutrient solutions contained 20 mg P/liter as $Ca(H_2PO_4)_2 \cdot H_2O$, 395 mg Mg/liter as $MgSO_4$ and $MgCl_2 \cdot 6H_2O$, and 4 mg Fe/liter as Sequestrene iron. KNO_3, KCl, $Ca(NO_3)_2 \cdot 4H_2O$, and $CaCl_2 \cdot 2H_2O$ were used in amounts required to provide variable NO_3 concentration while keeping K and Ca concentrations constant at 116 mg/liter and 168 mg/liter, respectively. Thus, variations in nitrate concentrations were balanced with chloride. Composition of the minor element solution was the same as described previously (20). pH was adjusted to 6.5 using NaOH.

Plants for Experiment 1 were grown in a greenhouse during April and May and fluorescent and incandescent lamps were used to supplement sunlight with about 200 $\mu E\ m^{-2}s^{-1}$ (400–700 nm). Plants for Experiment 2 were grown outdoors on benches about 70 cm above ground level during June and July. There was no alteration of photoperiod or supplementation of sunlight. Twenty plants were grown per pot (25 cm diameter) so that there was some competition among plants (overlap of leaf blades) beginning about 30 days after planting. Experimental designs were completely random and when pots (2 or 3 pots/sample) were taken for a harvest, the remaining pots were spaced equidistantly on the benches. Nutrient solutions were supplied once or twice per day beginning 3 or 4 days after planting using an automatic irrigation

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system. Sufficient solution was supplied to each pot to flush old solution through a hole at the bottom of each pot.

Extraction and Analysis. Five to seven nodulated roots were used for acetylene reduction assays using 10% (v/v) C_2H_2 and methods described previously (20). Nodules were removed from roots, weighed and counted, and then these nodules and roots were discarded.

For carbohydrate analyses, samples consisted of 4 to 5 g of nodules from 6 to 8 plants (0-N and 30-N treatments) or 15 to 20 plants (100-N treatment). Nodules and other plant parts were weighed and immediately ground in 95% (v/v) ethanol. After centrifugation, residue was reextracted four times with 75% ethanol. Combined extracts were dried *in vacuo*, dissolved in water, and stored at 2 C with a few drops of chloroform. After the last ethanol extraction, residue in centrifuge tubes was dried under a stream of air, quantitatively transferred to a mortar, and pulverized. Weighed portions of this residue were used for analysis of starch by enzymic digestion and analysis of glucose (22). Carbohydrate composition of ethanol extracts was determined by GLC as described previously (21) except that a Varian (Palo Alto, CA) 3700 gas chromatograph was used.

RESULTS

The effect of nitrate on nitrogenase, largely independent of the effect on growth, can be demonstrated by growing plants without nitrate and supplying nitrate after nodules are well developed. This effect is illustrated in Table I which also shows that the effect of nitrate on nitrogenase activity is at least partly reversed by withdrawing nitrate. Plants harvested after 4 to 7 days exposure to nutrient solution containing 150 mg N/liter showed an 80% decline in nodule activity relative to control plants (Table I). When nitrate was withdrawn, nodule activity increased to near the control level. Glucose concentration in nodules on plants supplied with nitrate was slightly lower than glucose concentration in control nodules at harvests of 48, 51, and 55 days after planting. However, sucrose concentration was higher in treated nodules than in control nodules at these same harvest times (Table I). Maltose and trehalose concentrations showed no consistent response to nitrate treatments (data not shown). Paau and Cowles (13) found little change, on the average, in sucrose concentration of soybean nodules after plants were supplied with nitrate. However, glucose concentrations increased initially to levels 4-fold above control nodules but, after 6 days, declined to levels below control nodules (13).

Effects of short term exposure to nitrate were not pursued since interest was in testing the idea that major reductions in nodule weight induced by continuous exposure to nitrate are due to reduced carbohydrate concentration in nodules. In Experiment 2, soybean plants were grown with or without nitrate and examined after 5 or 6 weeks. With soybeans, unlike some other legumes, continuous exposure to nitrate has a much greater effect on nodule weight than on nodule number or specific acetylene reduction activity (Table II). These effects have previously been reported by others (7, 9, 15).

Fresh weights are shown in Table II to document the effect of nitrate on growth rates of plants. The effect of nitrate on stem plus petiole weight was greater than the effect on roots and leaf blades. While nitrate-grown plants were clearly larger, plants grown without nitrate were vigorous and did not exhibit nitrogen deficiency. There were statistically significant but quantitatively small negative effects of nitrate (100-N) on glucose, sucrose, trehalose, and maltose at harvest 1 and on maltose and fructose at harvest 2 (Table II). It is important to note that the effects of nitrate on carbohydrate were small relative to the large effects on nodule weight/plant. Starch concentration was markedly increased in nitrate-grown nodules harvested 42 days after planting.

The 30-N treatment was included and is shown in Table II because moderate concentrations of nitrate generally have no effect or a small positive effect on soybean nodule growth and activity (7, 9, 15). This response to moderate NO_3 was confirmed here. The effect of the 30-N treatment on carbohydrates was not uniform among compounds or between harvests. For example, 30-N nodules contained less sucrose than 0-N nodules at harvest 1 but more sucrose than 0-N nodules at harvest 2. Response of starch concentration to nitrate was similar to the response of sucrose (Table II).

Carbohydrate composition of roots was also determined in this experiment because the original carbohydrate deprivation hypothesis was based on the observation that reducing sugar concentration of roots is lowered by long-term exposure to nitrate (12, 24). By comparing 0-N and 100-N treatments, the effects of nitrate on glucose and fructose concentration were evident in roots (Table III); the effect of nitrate on fructose was especially pronounced. The effect of nitrate on sucrose concentration in roots was large at harvest 1 but was not significant at harvest 2.

The generally greater effect of nitrate on root carbohydrate than on nodule carbohydrate concentration was emphasized by calculating ratios of concentration in nodules *versus* roots (Table III). Nitrate increased the ratios for fructose and sucrose and had little

Table I. *Acetylene Reduction Activity and Carbohydrate Concentration in Soybean Nodules as Influenced by Alteration of N Concentration in Nutrient Solution from 0 to 150 mg N as NO_3/liter*

After the initial observations 44 days after planting, NO_3 concentration supplied to treated plants was varied for periods of 7 days during which additional observations were made. Control plants were supplied with solution lacking nitrate throughout the experiment. Before switching from 150-N to 0-N solution, all pots were thoroughly flushed with tap water. Fructose concentration in these samples was so low and *chiro*-inositol so high that fructose could not be accurately quantified (21). Data are averages of two replicates except for 44 days after planting (four reps) (experiment 1).

Harvest Date or days after planting	Nutrient Solution Treatment	Duration of Treatment	Acetylene Reduction Activity		Carbohydrate concentrations					
					Cyclitols[a]		Glucose		Sucrose	
			Control	Treated	Control	Treated	Control	Treated	Control	Treated
	mg N/l	*days*	*µmol/g fresh wt·h*				*mg/g fresh wt*			
44			13.6		1.93		0.64		0.62	
48	150	4	13.8	2.5	2.15	1.92	0.46	0.35	0.89	1.03
51	150	7	13.2	2.9	2.45	2.67	0.56	0.36	1.92	2.23
55	0	4	13.7	5.4	3.32	2.54[b]	0.49	0.32	1.09	1.23
58	0	7	14.8	12.9	2.97	4.21[b]	0.58	0.65	2.30	3.80

[a] Sum of D-pinitol, D-*chiro*-inositol, and *myo*-inositol.

[b] Nearly all of this difference from the control could be accounted for by D-pinitol.

Table II. *Effect of Nitrate Concentration in Nutrient Solution on Fresh Weight, Acetylene Reduction Activity, and Carbohydrate Composition of Soybean Nodules at two Stages of Growth*

Plants were grown outdoors in sand culture during summer months. Mean and SE (superscript) of four replicates are shown (experiment 2).

	36 Days after Planting			42 Days after Planting		
	NO_3-N (mg N/1)			NO_3-N (mg N/1)		
	0	30	100	0	30	100
Fresh weight, g/plant						
Leaf blades	$3.15^{0.14}$	$3.38^{0.10}$	$3.48^{0.13}$	$4.04^{0.16}$	$4.70^{0.19}$	$5.09^{0.50}$
Stems + petioles	$3.24^{0.14}$	$4.05^{0.11}$	$5.15^{0.28}$	$4.14^{0.16}$	$5.65^{0.14}$	$6.74^{0.79}$
Roots	$3.22^{0.19}$	$3.09^{0.24}$	$3.75^{0.18}$	$4.25^{0.32}$	$4.66^{0.42}$	$5.60^{0.28}$
Nodule fresh wt, g/plant	$0.60^{0.01}$	$0.54^{0.03}$	$0.18^{0.01}$	$0.78^{0.03}$	$0.79^{0.02}$	$0.28^{0.01}$
Number of nodules/plant	$50.2^{1.3}$	$53.0^{3.3}$	$35.5^{1.4}$	$49.9^{1.7}$	$57.8^{5.2}$	$37.3^{1.1}$
Acetylene reduction, μmol/g fresh wt·h	$10.1^{1.2}$	$8.7^{0.5}$	$7.5^{1.1}$	$20.4^{2.2}$	$20.2^{0.8}$	$13.7^{0.5}$
Carbohydrate concentration, mg/g fresh wt						
D-pinitol	$1.27^{0.07}$	$1.67^{0.09}$	$1.59^{0.09}$	$1.39^{0.12}$	$1.84^{0.16}$	$1.78^{0.26}$
D-*chiro*-inositol	$1.07^{0.04}$	$1.11^{0.09}$	$1.30^{0.10}$	$1.03^{0.13}$	$1.18^{0.07}$	$1.43^{0.09}$
myo-inositol	$0.98^{0.05}$	$0.92^{0.06}$	$0.71^{0.07}$	$0.98^{0.05}$	$0.92^{0.06}$	$0.71^{0.07}$
Fructose	$0.093^{0.011}$	$0.073^{0.015}$	$0.087^{0.007}$	$0.149^{0.033}$	$0.156^{0.036}$	$0.083^{0.005}$
Glucose	$0.90^{0.05}$	$0.88^{0.08}$	$0.68^{0.07}$	$0.81^{0.07}$	$0.87^{0.07}$	$0.71^{0.02}$
Sucrose	$1.72^{0.17}$	$1.32^{0.15}$	$1.28^{0.18}$	$1.65^{0.02}$	$2.65^{0.48}$	$1.85^{0.24}$
Maltose	$0.24^{0.02}$	$0.20^{0.01}$	$0.12^{0.02}$	$0.18^{0.01}$	$0.22^{0.01}$	$0.12^{0.01}$
α,α-Trehalose	$0.13^{0.02}$	$0.10^{0.01}$	$0.08^{0.01}$	$0.12^{0.03}$	$0.13^{0.01}$	$0.08^{0.01}$
Starch concentration, glucose equivalents, mg/g dry wt	$44.3^{1.4}$	$33.9^{1.2}$	$44.5^{2.2}$	$48.8^{2.5}$	$73.3^{1.8}$	$74.1^{2.5}$

Table III. *Effect of Nitrate Concentration in Nutrient Solution on Carbohydrate Composition of Soybean Roots and on the Ratio of Carbohydrate Concentration in Nodules/Carbohydrate Concentration in Roots*

Mean and SE (superscript) are shown (experiment 2).

	36 Days after Planting			42 Days after Planting		
	NO_3-N (mg N/1)			NO_3-N (mg N/1)		
	0	30	100	0	30	100
Carbohydrate concentration, mg/g fresh wt						
D-pinitol	$0.43^{0.05}$	$0.38^{0.03}$	$0.52^{0.05}$	$0.62^{0.03}$	$0.52^{0.10}$	$0.49^{0.05}$
Fructose	$0.49^{0.05}$	$0.46^{0.10}$	$0.27^{0.04}$	$1.23^{0.19}$	$0.68^{0.05}$	$0.53^{0.09}$
Glucose	$0.34^{0.04}$	$0.33^{0.06}$	$0.25^{0.04}$	$0.75^{0.14}$	$0.55^{0.07}$	$0.57^{0.08}$
Sucrose	$0.55^{0.06}$	$0.35^{0.03}$	$0.28^{0.05}$	$0.91^{0.08}$	$0.71^{0.08}$	$0.84^{0.13}$
Starch concentration, glucose equivalents, mg/g dry wt	$20.2^{0.5}$	$17.4^{0.3}$	$19.4^{0.7}$	$22.6^{1.0}$	$22.2^{0.8}$	$34.4^{3.1}$
[Compound in Nodule] ÷ [Compound in root]						
D-pinitol	$3.0^{0.3}$	$4.4^{0.2}$	$3.1^{0.3}$	$2.1^{0.2}$	$3.8^{0.5}$	$3.7^{0.5}$
Fructose	$0.19^{0.02}$	$0.16^{0.01}$	$0.33^{0.04}$	$0.12^{0.04}$	$0.23^{0.05}$	$0.18^{0.05}$
Glucose	$2.9^{0.6}$	$2.8^{0.3}$	$2.8^{0.4}$	$1.0^{0.2}$	$1.7^{0.2}$	$1.4^{0.2}$
Sucrose	$3.1^{0.2}$	$3.7^{0.2}$	$3.8^{0.5}$	$1.9^{0.4}$	$3.7^{0.5}$	$2.3^{0.3}$

effect on the ratio for glucose. The low ratio for fructose, relative to other compounds, may indicate relatively rapid utilization of fructose in nodules (21).

It is possible to obtain greater effects of nitrate on nodule carbohydrates than the effects shown in Table II. Effects of nitrate on carbohydrate concentration are magnified when plants are grown with light energy levels less than full sunlight. Experiment 2 (Table II) was conducted during a period when mean daily irradiance was 500 g-cal m^{-2} day^{-1} and only 17% of the days during the plant growth period had less than 300 g-cal m^{-2} (*i.e.*, were overcast).

In another experiment, similar in design to experiment 2, mean irradiance during the plant growth period was only 379 g-cal m^{-1}day^{-1} and 35% of the days had less than 300 g-cal m^{-2}. The effect of nitrate on nodule carbohydrates in this "low light" experiment is shown in Table IV. The average sugar (glucose + fructose + sucrose) concentration in 100-N nodules was 74% of the average sugar concentration in 0-N nodules (Table IV). In the "high light" experiment (Table II) the average sugar concentration in 100-N nodules was 88% of the average sugar concentration in 0-N nodules. For starch, 100-N nodules had 79% of the concentration in 0-N nodules in the "low light" experiment (Table IV) and 127% of the concentration in 0-N nodules in the "high light" experiment (Table II).

The sensitivity of sugar concentrations in nodules to light energy is also illustrated by the variation in sugar concentrations among harvests in the "low light" experiment (Table IV). Sugar and starch concentrations in nodules appeared to be related to the light energy available to plants on the day preceding the harvest. Thus, while nitrate (100-N) reduced sugar and starch concentrations by about 25%, environmental factors (probably light) had a much greater influence on nodule carbohydrate.

DISCUSSION

The carbohydrate deprivation hypothesis states that consumption of carbohydrate to support nitrate assimilation results in a decline in the amount of carbohydrate supplied to nodules. The implication of the hypothesis is that carbohydrate concentration

Table IV. *Relationship between Incident Light Energy and Carbohydrate Concentration in Soybean Nodules Grown with or without NO_3 in Nutrient Solution and Harvested at Five Stages of Growth*

Plants were grown outdoors during July and August with no supplemental light. Nodule weight/plant was reduced 77%, on the average, by the 100-N treatment. Plants were grown during a period of low mean daily irradiance relative to Experiment 2 (see text). Average of two replicates is shown.

Plant age or days after planting	Sucrose + Glucose + Fructose Concn		Starch Concentration		Light Energy Day Before Harvest
	NO_3-N concn mg N/l		NO_3-N concn mg N/l		
	0	100	0	100	
	mg/g fresh wt		*mg/g dry wt*[a]		*g-cal/m²*
25	3.36	2.92	129	88	584
31	1.21	0.95	47	45	406
37	1.45	0.95	31	31	216
43	2.20	1.16	64	35	393
50	4.39	3.35	94	92	540
Mean	2.52	1.87	73	58	
100-N as % of O-N	74%		79%		

[a] mg Glucose equivalents/g dry residue after extraction with 75% ethanol.

in nodules will be lowered as a result of reduced supply, thereby inhibiting nodule growth and function. Plants supplied with nutrient solution containing 100 mg N/liter had 70% less nodule mass and slightly reduced specific acetylene reduction activity relative to 0-N or 30-N plants. There is little doubt that the supply of carbohydrate to 100-N nodules was reduced, relative to 0-N nodules. But the fact that there was little effect of nitrate on carbohydrate concentration in 100-N nodules is taken as evidence that nodule growth and N_2-fixing activity were not impaired as a result of the reduced carbohydrate supply.

Since 90% or more of the nitrate reductase activity in soybean plants is located in the leaf blades (8, 16), one might predict a major influence of nitrate on leaf blade carbohydrate. Analysis of leaf blades from some of the experiments reported here has, in fact, revealed 40 to 50% reductions in sugar (glucose + fructose + sucrose) concentrations in leaf blades from 100-N plants relative to 0-N plants (data not shown). (Nitrate did not reduce starch concentration in leaf blades [data not shown]). However, the central question is whether or not nitrate treatments result in a concomitant lowering of carbohydrate concentration in nodules thereby lowering their rate of growth and N-fixing activity.

The effect of nitrate on N-fixing activity, as revealed by short term exposure of plants to high nitrate concentrations (Table I), was associated with a decline in glucose concentration of 0.15 mg/g fresh weight. However, sucrose concentration in nodules increased by about 0.23 mg/g fresh weight in response to nitrate. Changes in glucose and sucrose concentrations were small relative to the large (4-fold) effects of nitrate on nitrogenase activity. These results are in agreement with an experiment where a 4-fold increase in CO_2 concentration supplied to pea plants did not delay the decline in acetylene reduction activity of nodules which was induced by nitrate (2).

The effect of nitrate on nodule growth, as revealed by long term exposure of plants to nitrate in nutrient solution (Table II), was also associated with a small decline in glucose concentration in nodules. Sucrose and fructose concentrations declined in response to nitrate at one harvest but not at the other (Table II). One could argue that glucose is a more readily available source of carbon than sucrose to support nodule growth and activity. However, sucrose is the principal form of reduced carbon translocated in the soybean plant (1) and its concentration in nodules is correlated with acetylene reduction activity in certain types of experiments (3, 23). Thus, until more detailed information on compartmentation and metabolism of carbohydrates in nodules becomes available, it is reasonable to assume that sucrose, glucose, and fructose are equivalent in their ability to support nodule growth and activity. If this assumption is correct, the fact that a 70% decline in nodule weight/plant caused by 100-N treatment was accompanied by only a 12% decline in sugar concentration in nodules makes it seem unlikely that lack of carbohydrate was the cause of reduced nodule growth. It should be added that 100-N nodules, in spite of slightly reduced carbohydrate concentration, contained higher carbohydrate concentration than other plant organs.

The role of the cyclitols in nodule function is still not clear. Data for the cyclitols were included in the tables because the cyclitols are major carbohydrate components of soybean nodules, because their accumulation in nodules coincides with the onset of N fixation (21), and because the concentration of D-pinitol is correlated with nitrogenase activity (23). Nitrate treatments generally resulted in increases in D-pinitol and D-*chiro*-inositol concentrations in nodules (Table II) and roots (Table III), while *myo*-inositol concentration in nodules decreased in response to nitrate (Table II).

The decline in glucose and fructose concentrations in roots (Table III) is in agreement with the results of Strowd (24) and Orcutt and Wilson (12) which showed a decline in reducing sugar concentration in soybean roots in response to nitrate. Using ferricyanide analysis (22) a decline in reducing sugar concentration of our nitrate-grown roots was also observed (data not shown). Ferricyanide analyses indicated a greater effect of nitrate on reducing sugar concentration of roots (30% decline) than on reducing sugar concentrations of nodules (13% decline). Thus, while these results confirm the early work on the effect of nitrate on reducing sugars in roots, the sugar concentration of nodules cannot be inferred from the sugar concentration of roots.

The greater effect of nitrate on root sugars than nodule sugars is shown more clearly by the finding that [sucrose] nodule/[sucrose] root and [fructose] nodule/[fructose] root ratios increased slightly when plants were grown with nitrate (Table III). The ratios in Table III are also more convincing than reducing sugar results because ferricyanide analyses can be influenced by reducing substances other than carbohydrate in extracts.

One of the main sources of support for the concept that nitrate inhibits the growth and activity of nodules by limiting the availability of carbohydrates comes from studies showing a reduction in the proportion of ^{14}C in nodules from $^{14}CO_2$ assimilated by shoots when plants are supplied with nitrate (10, 14, 18). These results may be explained without invoking a carbohydrate deprivation hypothesis if nitrate inhibits nodule growth and/or function by some mechanism unrelated to carbohydrate and lowers the demand for carbohydrate in nodules. If demand for carbohydrate is lowered in the presence of nitrate, one would expect a smaller proportion of $^{14}CO_2$ assimilated to be transported to nodules.

It is not so easy to explain the results of Fred and Wilson (5) who showed that CO_2-enrichment of clover plants partially counteracted the reduction in nodule weight caused by nitrate. Not all of the relevant data were reported for these experiments (5) so that one can only speculate that CO_2-enriched plants were larger and that nodule weight as per cent of total plant weight was not altered by CO_2 enrichment and was markedly reduced by nitrate. It should also be restated that Chen and Phillips found that 4-fold CO_2 enrichment did not prevent the negative effect of nitrate on acetylene reduction activity of pea nodules (2). However, as already implied in this paper, short term effects of nitrate on nitrogenase and long term effects of nitrate on nodule growth may or may not have a common mechanism.

Results reported here do not support the idea that nitrate inhibits nodule growth and N-fixing activity by reducing the accumulation of carbohydrate in nodules. However, the results do not rule out the possibility that nitrate somehow interferes with the catabolism of carbohydrates in nodules (19). It remains possible that while nodules may be able to obtain sufficient carbohydrate in the presence of nitrate, their ability to use that carbohydrate might be restricted by some influence of nitrate on carbohydrate transport or metabolism within the nodule.

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