Plant Physiol. (1975) 55, 923–927

Effects of Calcium on the Photosynthesis of Intact Leaves and Isolated Chloroplasts of Sugar Beets[1]

Received for publication August 26, 1974 and in revised form January 8, 1975

NORMAN TERRY AND ROBERT P. HUSTON
Department of Soils and Plant Nutrition, University of California, Berkeley, California 94720

ABSTRACT

Effects of calcium on photosynthesis in sugar beets (*Beta vulgaris* L. cv. F58-554H1) were studied by inducing calcium deficiency and determining changes in CO_2 uptake by attached leaves, electron transport, and photophosphorylation by isolated chloroplasts, and CO_2 assimilation by ribulose diphosphate carboxylase extracts. Calcium deficiency had no significant effect on leaf CO_2 uptake, photoreduction of ferricyanide, cyclic or noncyclic ATP formation of isolated chloroplasts, or on ribulose diphosphate carboxylase CO_2 assimilation, when the rates were expressed per unit chlorophyll. When expressed per unit leaf area CO_2 uptake increased by about 15% in low calcium leaves. The most noticeable effect of calcium deficiency was reduction in leaf area: low calcium had no effect on dark respiratory CO_2 evolution, on leaf diffusion resistance, or on mesophyll resistance to CO_2. We concluded that only small amounts of calcium are required for normal photosynthetic activity of sugar beet leaves.

Calcium, as an insoluble salt of pectic acid, may act as a binding agent in the middle lamella between primary cell walls. More recently, it has been suggested that Ca may be required for the maintenance and formation of cellular membranes (9). Membrane permeability may be increased if there is an insufficient supply of Ca (13, 22). Calcium may be required for normal root and root hair development (17), cell multiplication and division (4), carbohydrate translocation (8), protein and nucleic acid synthesis (6, 15), nitrate uptake and assimilation (11), and auxin transport (5), and may have some role in abscission (12).

In view of the postulated role of Ca in cell membranes one might anticipate an effect of Ca on the structure and function of such highly membrane-structured organelles as chloroplasts and mitochondria. Ca deficiency has been shown to damage chloroplast lamellae (14) and to diminish rates of photosynthesis of isolated chloroplasts (16) and intact leaves (3). However, Baszynski *et al.* (2) found photosystem I and photosystem II activity in corn chloroplasts to be affected very little by Ca, whereas Repka *et al.* (14) found Ca deficiency diminished photosynthetic CO_2 uptake by only 15%. Calcium deficiency has been shown to affect respiration (3, 6, 24). Further investigation appears to be necessary to assess more fully the extent of the effect of Ca on photosynthesis and respiration. In the present investigation this has been done by following changes in the CO_2 exchange of intact leaves and in the photosynthetic activities of isolated chloroplasts and RuDP[2] carboxylase extracts with the onset of Ca deficiency.

MATERIALS AND METHODS

Plant Culture and Analysis. Sugar beets (*Beta vulgaris* L. cv. F58-554H1) were cultured hydroponically in growth chambers to the 10-leaf stage, 28 days after planting, and Ca deficiency was induced by reducing the Ca concentration of the culture solution to one-fifth of that of control plants for 19 days (experiment I) or 20 days (experiment II). In experiment III Ca was totally withheld for 21 days. Plants were harvested at 2- or 3-day intervals beginning on the first day of treatment. Measurements of leaf mineral contents and photosynthetic activities of isolated chloroplasts and RuDP carboxylase extracts were carried out at each harvest in all three experiments but gas exchange parameters were determined only in experiments I and II.

Details of the procedure followed in the culture of plants, in the determination of gas exchange parameters of individual attached leaves, and in the estimation of the contents of leaf minerals are as outlined in earlier papers (18, 19, 21). The composition of the culture solutions for experiments I and II (expressed in mmole/l) was: 0.5 $Ca(NO_3)_2$, 4 $NaNO_3$, 0.5 KH_2PO_4, 2.5 KNO_3, 1 $MgSO_4$, and 0.5 NaCl, and, in mg/l, 0.25 B, 0.25 Mn, 0.025 Zn, 0.01 Cu, and 0.005 Mo. Iron was added as ferric-sodium ethylene diamine tetraacetate complex to give 2.5 mg of Fe/l. In experiment III the $Ca(NO_3)_2$ was replaced with 1 mmole of $NaNO_3$/l. The culture solution used for control plants differed in composition in that it contained 2.5 mmole/l of $Ca(NO_3)_2$ and no $NaNO_3$.

PROCEDURES USED FOR ISOLATED CHLOROPLASTS STUDIES

Sampling. Two or three leaves of one plant were sampled at each harvest. The gas exchange analysis was usually carried out on Monday, Wednesday, and Friday of each week while the leaves for chloroplast studies were harvested Monday and Wednesday. The leaf chosen for chloroplast study was comparable to the leaf chosen for gas exchange analysis but was taken from a different plant; it was the largest (150–300 cm^2 for a control leaf) of the newly expanded apical leaves and is arbitrarily designated leaf 2. When two leaves were sampled, the leaf immediately preceding leaf 2 in order of formation was selected in addition (leaf 1), and when three leaves were sampled the leaf following leaf 2 was selected (leaf 3).

[1] This work was supported by the Beet Sugar Development Foundation.

[2] Abbreviation: RuDP: ribulose 1,5-diphosphate.

Following measurement of leaf area, the leaf was subsampled for eventual estimation of Chl content and for determination of RuDP carboxylase activity by taking 20 discs, each 0.5 cm^2 in area, at random over the leaf surface (excepting the mid-rib and main veins). The remaining leaf was separated into two halves along but excluding the mid-rib. The two halves were chopped into 1-cm square pieces and mixed, and a 3-g subsample was taken for chloroplast extraction. The remainder of the leaf was used for determination of mineral content after drying at 70 C and weighing. All fresh leaf material was stored on ice in the dark.

Chloroplast Isolation. The 3-g leaf sample was ground in 15 ml of an isotonic preparative solution using a precooled mortar and pestle and sand. The composition of the preparative solution was 0.5 M NaCl, 0.01 M ascorbic acid, and 0.04 M Tricine (the pH was 8.2 at 20 C). After the slurry had been filtered through a silk filter, it was centrifuged at 200*g* for 1 min at 0 C to sediment sand and cellular debris. The supernatant was decanted and the chloroplasts were separated by centrifugation at 2500*g* for 1 min. This supernatant was poured off and the chloroplasts were resuspended in 0.2 ml of extraction mixture with the aid of a stirring rod wrapped in absorbent cotton and eventually diluted up to a volume of 2 ml. The Chl content of the mixture plus chloroplasts was quickly determined (see below), adjusted to about 0.5 mg of Chl/ml, and the photosynthetic activity of the chloroplasts determined within 1 hr.

Chlorophyll Estimation. One-tenth milliliter of chloroplast suspension was diluted to 10 ml with 80% acetone, filtered and the Chl ($a + b$) absorbance determined at 652 nm by means of a Beckman DU spectrophotometer according to the method of Arnon (1). The total Chl content of the leaf was obtained by grinding 10 leaf discs in a tissue homogenizer with 80% acetone, diluting to 25 ml, and determining the absorbance as before.

Noncyclic Photophosphorylation. Chloroplast suspension (0.05 ml) was pipetted into 0.45 ml of assay solution contained in a 10 × 75 mm test tube and illuminated at saturating intensities (5000 ft-c) for 4 min at 20 C using two 300 w G.E. reflector flood lamps. The final reaction medium after addition of the chloroplast suspension to the assay solution contained 0.1 M Tricine (pH 8.2), 2 mM $MgCl_2$, 2 mM $K_2H^{32}PO_4$, 2 mM ADP, and 4 mM $K_3Fe(CN)_6$. Immediately following illumination, 1 ml of killing mixture (0.6 M Na_2SO_4 in 0.9 M perchloric acid) was pipetted into the chloroplast suspension. The samples were stored at 0 C to await estimation of ATP within 24 hr.

Estimation of ATP. Bromine water (0.03 ml) and 1.5 ml of ammonium molybdate reagent (50 volumes of 4% (w/v) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1.2 volumes hexanol, 40 volumes methanol made up to 100 volumes with H_2O) were pipetted into 1.5 ml of killed chloroplast suspension and mixed. A 0.5-ml aliquot was dried and the total ^{32}P (^{32}Pi + ^{32}ATP) counted. The remainder was poured through a treated celite column (see (7)) to remove the Pi, and a 0.5-ml aliquot of the solution containing ^{32}ATP was dried and counted.

Photoreduction of Ferricyanide. One-tenth milliliter of chloroplast suspension was pipetted into 0.9 ml of assay solution, shaken, and transferred into a 2-mm glass cuvette by syringe. The cuvette was placed in a Beckman Model DU monochromator which supplied a measuring beam of 420 nm and which was equipped with a Gilford Model 220 absorbance measuring attachment. The chloroplasts were illuminated with monochromatic light of 650 nm from a 1200 w DHT projection lamp and the change of absorbance which occurred on photoreduction of the ferricyanide was monitored over a period of 3 to 6 min. The rate of photoreduction was determined using the molar extinction coefficient for ferricyanide,

$$\epsilon_{420}\ cm^{-1}\ mM = 1.02.$$

For further details of this procedure see McSwain (10).

Cyclic Photophosphorylation. Chloroplast suspension (0.05 ml) was pipetted into 0.45 ml of assay solution on ice and illuminated at 5000 ft-c for 3 min at 20 C. One milliliter of killing mixture was then pipetted into each tube and the samples were stored at 0 C in the dark. The reaction medium was the same as for noncyclic photophosphorylation except that it contained 50 μM phenazine methosulfate in place of the $K_3Fe(CN)_6$. ATP was determined as before.

EXTRACTION AND ESTIMATION OF RUDP CARBOXYLASE

A chopped leaf sample of from 150 to 200 mg (10 leaf discs) was ground with 10 ml of preparative solution in a tissue homogenizer, centrifuged at 30,000*g* for 20 min at 0 C, and the supernatant containing the enzyme extract collected in a chilled test tube. The composition of the preparative solution was 40 mM tris-HCl, 10 mM $MgCl_2$, 0.25 mM EDTA, 5 mM sodium ascorbate, and 5 mM dithiothreitol; the pH was adjusted to 8 with N KOH. The activity of the RuDP carboxylase was determined by measuring the rate of assimilation of $H^{14}CO_3^-$ by 0.05 ml of the enzyme extract in the presence of RuDP contained in 0.35 ml of assay solution over a period of 5 min at 30 C. The composition of the reaction medium after addition of the enzyme to the assay solution was 60 mM tris-HCl (pH 8), 6 mM $MgCl_2$, 0.2 mM EDTA, 0.3 mM RuDP, and 50 mM $NaH^{14}CO_3$ (0.1 μCi/μmole). Enzyme activity was terminated by addition of 0.1 ml of 6 N acetic acid, and the incorporated ^{14}C counted in a gas-flow counter.

RESULTS

Leaf Minerals. Five days after the Ca supply was reduced to one-fifth (referred to here as Ca cut-off), the Ca concentration of newly unfolded leaves decreased from 650 to 200 meq Ca kg^{-1} dry weight (Fig. 1). The sodium concentration increased from about 250 to 650 meq kg^{-1} over the same period (Fig. 1), which suggests that Na was taken up by the leaf as a substitute cation for Ca as the supply of Ca became depleted. Sodium, which in these experiments was supplied at the rate of 4.5 or 5.5 mmole/l, was apparently taken up exclusively since the concentrations of the other major cations, K and Mg, decreased with time (Fig. 1). The concentrations of Mn, Cu, Zn, and P also decreased with time but the concentration of Fe was unchanged (Fig. 1). The decreases in leaf nutrient concentrations were probably due to the rapid growth of the plants, the demand for nutrients outstripping supply both in control and in Ca-deficient plants. Visible symptoms of Ca deficiency such as leaf cupping appeared about 8 days after Ca cut-off.

Leaf Chlorophyll. The Chl content per unit leaf area (y, μg Chl cm^{-2}) increased slightly as the leaf Ca concentration (x, meq Ca kg^{-1}) decreased, the regression of y on x ($y = 50.6 - 0.017\ x$, combined data from experiments I and II) being significant at $P = 0.05$. One possible explanation of the increased Chl content per unit area of leaf is that low Ca leaves expanded to smaller areas than control leaves while continuing to synthesize Chl at high rates. It was apparently not due to an increase in leaf thickness since the ratio of leaf weight (fresh or dry weight) to leaf area did not change significantly with leaf Ca.

CO_2 Exchange. After reducing the Ca supply to one-fifth, photosynthetic rates in normal and O_2-free air apparently increased to a greater extent in low Ca leaves than in control

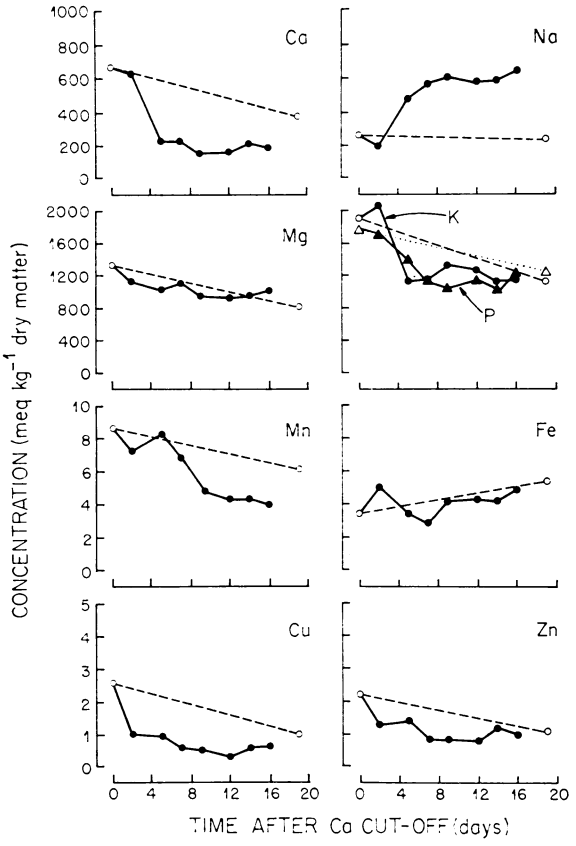

FIG. 1. Effects of Ca deficiency on the concentrations of various elements in the leaf blade. Data are for experiment 1 in which the supply of Ca was reduced to one-fifth at cut-off. For convenience the concentrations of Fe, Cu, and Mn are expressed in terms of the element in its divalent form. Control plants (—○—).

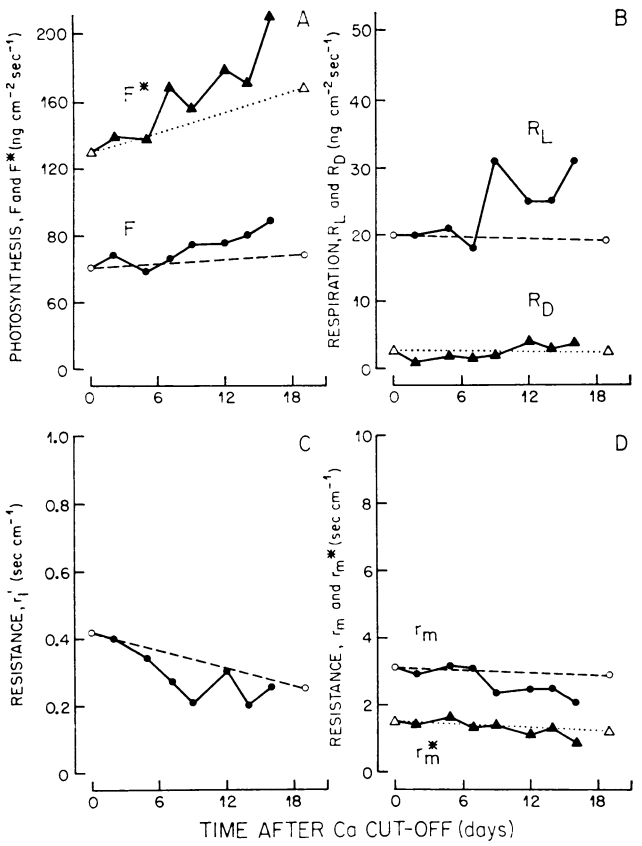

FIG. 2. Effects of Ca deficiency on various leaf gas exchange parameters. Data are for experiment 1 in which the Ca supply was reduced to one-fifth at cut-off. A: Changes in rate of photosynthetic CO_2 uptake in O_2-free air, F^*, and in normal air (21% O_2), F; B: changes in rates of respiratory CO_2 evolution in the light, R_L, and dark, R_D; C: changes in leaf diffusion resistance for water vapor; r_l'; D: changes in CO_2 mesophyll resistance in O_2-free air, r_m^*, and in normal air, r_m. The data for F, F^*, and r_l' were determined at 35 mw cm^{-2} visible radiation, 25° C, and at an ambient CO_2 concentration of 300 ng cm^{-3}. Control plants (—○—, —△—).

leaves (Fig. 2A). If the data from experiments I and II are combined, then a regression of F, or F^*, with leaf Ca concentration, shows that there was an increase (significant at $P = 0.05$) in F or F^* with decrease in leaf Ca. If, however, the rate of photosynthesis is expressed per unit Chl instead of per unit area, the linear regression is not significant. Thus, as there was an increase in the Chl content per unit area of leaf with decrease in leaf Ca, the increases in photosynthetic rate with Ca deficiency were apparently due to an increased Chl content per unit area.

As the photosynthetic rate increased following the reduction in the supply of Ca, so did the light respiration rate, R_L (Fig. 2B). There was no significant change in dark respiration, R_D, following Ca cut-off (Fig. 2B). Mesophyll resistance, r_m and r_m^*, decreased slightly compared to the control (Fig. 2D), while leaf diffusion resistance, r_l', decreased in both the control and Ca-deficient plants (Fig. 2C).

Photosynthetic Activities of Isolated Chloroplasts and of Extracted RuDP Carboxylase. The rate of photoreduction of ferricyanide, the rate of formation of ATP by noncyclic photophosphorylation using ferricyanide as electron acceptor, and the rate of production of ATP in cyclic photophosphorylation using phenazine methosulfate as catalyst were not changed significantly with leaf Ca content or with leaf position. There was, however, considerable variability in measurements from day to day, the standard deviation being about ± 20 to 30% of the mean (Table I). We minimized the problem of day to day variability by measuring the activities of chloroplasts isolated from control leaves and Ca-deficient leaves at the same time. This procedure was used in experiment III in which we also withheld all Ca from the culture solution so as to obtain leaves very low in Ca.

Table I. *Photosynthetic Activities of Isolated Chloroplasts and Extracted RuDP Carboxylase*

Combined data for experiments I and II. An analysis of variance indicated that Ca deficiency had no significant effect on photosynthetic activities. The values (with standard deviations) shown in the table were therefore obtained by averaging the data from 12 leaves of different Ca concentrations in the case of leaf 1 and leaf 2 and from 4 leaves in the case of leaf 3.

	Photoreduction of Ferricyanide	Noncyclic ATP Formation	Cyclic ATP Formation	RuDP Carboxylase CO_2 Assimilation
	μmole Fe mg Chl^{-1} hr^{-1}	*μmole ATP mg Chl^{-1} hr^{-1}*	*μmole ATP mg Chl^{-1} hr^{-1}*	*μmole CO_2 mg Chl^{-1} hr^{-1}*
Leaf 1	530 ± 68	191 ± 32	359 ± 98	338 ± 51
Leaf 2	527 ± 122	185 ± 48	402 ± 163	346 ± 108
Leaf 3	481 ± 135	193 ± 50	386 ± 171	319 ± 37

The results of experiment III are shown in Figure 3. Very low leaf Ca concentrations were obtained, in some instances less than 5% of the Ca concentrations of control leaves. The

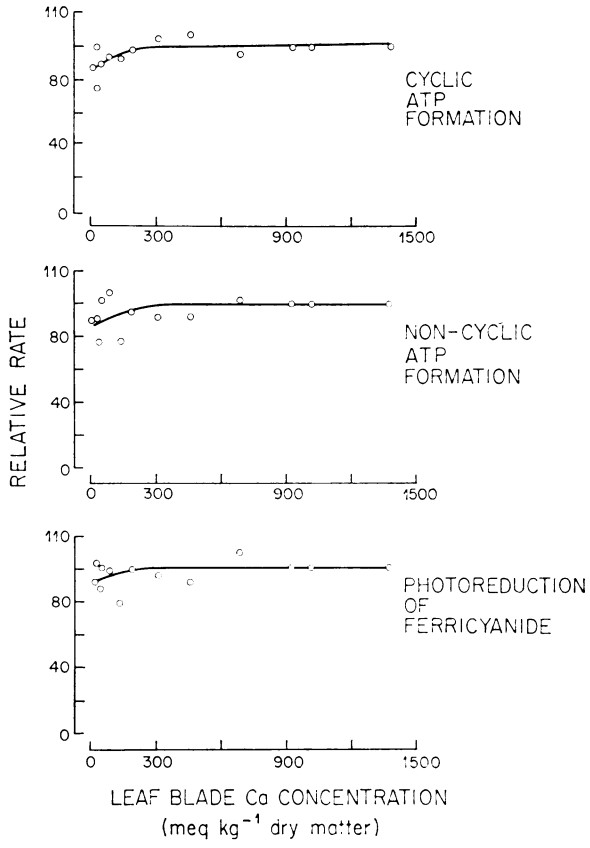

FIG. 3. Changes in rates of three different types of photosynthetic activity of isolated chloroplasts with leaf blade Ca concentration. Data are expressed relative to the control rates (= 100).

leaves were highly Ca-deficient in appearance; the leaf blade was severely cupped and very much reduced in size, some of the leaves being only 12 to 30 cm^2 in area. Despite these obvious symptoms of severe Ca deficiency and despite the low leaf Ca contents, there was no significant decrease at $P = 0.05$ in the rates of cyclic or noncyclic ATP formation, or in ferricyanide reduction. However, the data in Figure 3 could be taken to indicate that photosynthetic activities were beginning to be diminished by low Ca below 100 meq Ca kg^{-1}, even though this trend was not found to be significant.

The rate of CO_2 assimilation by the chloroplast RuDP carboxylase extract was about 320 to 350 μmoles of CO_2 mg^{-1} Chl hr^{-1} with CO_2 (HCO_3^-) supplied in saturating amounts (Table I). This compares with rates of CO_2 uptake which were about 140 to 180 μmoles of CO_2 mg^{-1} Chl hr^{-1} at ambient CO_2 levels of 300 ng of CO_2 cm^{-3} air which are considerably below saturation. There was no significant effect of leaf position or leaf Ca content on RuDP carboxylase activity.

DISCUSSION

The surprising feature of these results is that Ca deficiency, unlike any nutrient element deficiency we have studied, had no significant effect on photosynthesis when expressed per unit chlorophyll, no matter how severe the deficiency became. This applied to photosynthetic CO_2 uptake rates by intact attached leaves, the photoreduction of ferricyanide by isolated (broken) chloroplasts, cyclic anad noncyclic photophosphorylation by chloroplasts, and CO_2 assimilation by crude extracts of RuDP carboxylase. The leaf blade Ca concentration decreased from about 1000 to 100 meq Ca kg^{-1} dry matter with no effect whatever on photosynthetic activities, and some leaves reached as low as 17, 29, and 38 meq Ca kg^{-1} with areas of 29, 114, and 12 cm^2, respectively, and still retained 80 to 90% of the photosynthetic activities of the control. The principal effect of Ca deficiency was on leaf area which was considerably reduced, the leaves being severely cupped and distorted compared to the control leaves. The Chl content per unit area was increased slightly but significantly by Ca deficiency, presumably because Chl synthesis was less affected by Ca deficiency than leaf expansion. Ratios of area to leaf blade weight and of leaf fresh weight to dry weight were not changed by Ca deficiency.

So the effect of Ca on photosynthesis appears to be small compared to deficiencies of other elements such as phosphorus (18), manganese (20), or potassium (19). The actual requirement for Ca by higher plants for metabolic activities in general may be relatively small. Faust and Klein (6) showed that 90% of the Ca could be removed exchangeably from apple tissue without affecting its metabolism. Wallace *et al.* (23) found that if the concentrations of potentially toxic cations such as Mg, Cu, Fe, Zn, and Sr in the culture solution were kept low, Ca was needed in only relatively small amounts. In the present investigation leaves took up only Na in response to depletion in the external supply of Ca, partly because Na was supplied in large amounts. Concentrations of other leaf cations, Mg, Mn, Cu, Fe, and Zn either decreased or did not increase, so that toxic concentrations of other cations were not taken up in substitution for Ca as leaves became more Ca-deficient. Thus the apparent absence of any effect of Ca on photosynthesis and respiration may be because only small amounts of Ca are needed for the metabolic activities of sugar beet leaves, and because the experimental conditions (high Na supply) were such that other potentially toxic ions were not taken up in sufficient amounts to affect either photosynthesis or respiration.

Acknowledgments—We thank John Krall, Clifford Carlson, and Carlos Llano for excellent technical assistance, and Dr. John S. McFarlane for supplying the F_1 hybrid sugar beet seed, F58-554H1.

LITERATURE CITED

1. ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.
2. BASZYNSKI, T., J. BRAND, R. BARR, D. W. KROGMANN, AND F. L. CRANE. 1972. Some biochemical characteristics of chloroplasts from mineral-deficient maize. Plant Physiol. 50: 410–411.
3. BOTTRILL, D. E., J. V. POSSINGHAM, AND P. E. KRIEDEMANN. 1970. The effect of nutrient deficiencies on photosynthesis and respiration in spinach. Plant Soil 32: 424–438.
4. BURNSTRÖM, H. 1952. Studies on growth and metabolism of roots. VIII. Calcium as a growth factor. Physiol. Plant. 5: 391–402.
5. DELA FUENTE, R. K. AND A. C. LEOPOLD. 1973. A role of calcium in auxin transport. Plant Physiol. 51: 845–847.
6. FAUST, M. AND J. D. KLEIN. 1974. Levels and sites of metabolically active calcium in apple fruit. J. Amer. Soc. Hort. Sci. 99: 93–94.
7. HAGIHARA, B. AND H. A. LARDY. 1960. A method for the separation of orthophosphate from other phosphate compounds. J. Biol. Chem. 235: 889–896.
8. JOHAM, H. E. AND L. JOHANSON. 1973. The effect of sodium and calcium on the translocation of ^{14}C-sucrose in excised cotton roots. Physiol. Plant. 28: 121–126.
9. MARINOS, N. G. 1962. Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. Amer. J. Bot. 49: 834–841.
10. MCSWAIN, B. D. 1968. Quantum requirements and enhancement effects of photosynthetic electron transport in chloroplasts. Ph.D. thesis. University of California, Berkeley.
11. MORGAN, M. A., W. A. JACKSON, AND R. J. VOLK. 1972. Nitrate absorption and assimilation in ryegrass as influenced by calcium and magnesium. Plant Physiol. 50: 485–490.
12. POOVAIAH, B. W. AND H. P. RASMUSSEN. 1973. Calcium distribution in the abscission zone of bean leaves. Electron microprobe X-ray analysis. Plant Physiol. 52: 683–684.

13. REHFELD, D. W. AND R. G. 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.
14. REPKA, J., M. SARIC, J. MAREK, AND M. ZIMA. 1971. Effect of macroelement deficiency on structure of chloroplasts and productivity of photosynthesis in maize plants. Sov. Plant Physiol. 18: 938-943.
15. SINGKH, O. S. AND V. K. SHARMA. 1972. Alterations in growth and metabolism of potato plants by calcium deficiency. Plant Soil 36: 363-369.
16. SPENCER, D. AND J. V. POSSINGHAM. 1960. The effect of nutrient deficiencies on the Hill reaction of isolated chloroplasts from tomato. Aust. J. Biol. Sci. 13: 441-455.
17. TANAKA, Y. AND F. W. WOODS. 1973. Root and root hair growth of oats: replaceability of calcium. Can. J. Bot. 51: 1655-1659.
18. TERRY, N. AND A. ULRICH. 1973. Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol. 51: 43-47.
19. TERRY, N. AND A. ULRICH. 1973. Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol. 51: 783-786.
20. TERRY, N. AND A. ULRICH. 1974. Photosynthetic and respiratory CO_2 exchange of sugar beet leaves as influenced by manganese deficiency. Crop Sci. 14: 502-504.
21. TERRY, N., L. J. WALDRON, AND A. ULRICH. 1971. An apparatus for the measurement of carbon dioxide and water vapor exchange of attached sugar beet leaves. J. Amer. Soc. Sugar Beet Technol. 16: 471-478.
22. VAN STEVENINCK, R. F. M. 1965. The significance of calcium on the apparent permeability of cell membranes and the effects of substitution with other divalent ions. Physiol. Plant. 18: 54-69.
23. WALLACE, A., A. ELGAZZAR, AND S. M. SOUFI. 1968. The role of calcium as a micronutrient and its relationship to the other micronutrients. Trans. 9th Int. Congr. Soil Sci. 2: 357-366.
24. ZHOLKEVICH, V. N. AND I. L. SHIDLOVSKAYA. 1971. Changes of energy metabolism in *Triticum vulgare* Vill. under calcium deficits. Sov. Plant Physiol. 18: 967-972.