EFFECTS OF CALCIUM DEFICIENCY ON NITRATE ABSORPTION AND ON METABOLISM IN TOMATO[1]

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(WITH THREE FIGURES)

Introduction

This series of experiments was undertaken in an effort to determine the relation of calcium to the metabolism of carbohydrates and of nitrogenous compounds. Calcium is generally regarded as an essential element, and within recent years several workers (**10, 17, 46, 55**) have described a yellowing and a dwarfing of plants that had been grown in a deficiency of calcium, although they disagree as to the precise effect of such deficiency on the plant.

The tomato was used in the present experiments because it is well adapted to nutrient culture and because it has been used in so many experiments by different workers that a considerable knowledge of its nutrient requirements and metabolism is available.

Experimental methods

The experiments were carried on in the greenhouse at New Brunswick, New Jersey, during the early summer of 1930, with tomato plants of the variety Marglobe. On June 17 about 1,500 plants were selected for uniformity from 3,000 plants that had been grown for several weeks in sifted loam soil in four-inch pots. The plants were about 30 cm. in height, and were rather yellowish green in appearance; analysis showed them to be comparatively high in carbohydrates, but low in nitrogen (tables II, III, IV and V). The roots of each plant were washed free of soil, and the two lower leaves were removed; 300 of the plants were then used for initial

analysis, and the remaining 1,200 were transplanted for experimental treatments to washed quartz sand in new ten-inch clay pots, one or two plants to a pot. The pots were set in shallow enamel-ware pans and for the three weeks of the duration of the experiment all of the plants were subjected to nutrient treatments, each pot receiving daily two liters of nutrient solution. Twice a week each culture was thoroughly flushed with distilled water, and fresh nutrient solution was applied immediately.

The 1,200 plants were divided into four series according to the nutrient treatment which they received (see table I). One series was supplied with a complete nutrient solution that had been shown by previous experiments to be well adapted to the growth of tomato plants. The three other series received solutions lacking either calcium or nitrates or both. From time to time some of the plants were shifted from one nutrient treatment to another. The nutrient solutions employed all had a pH of about 4.7 when applied. After the solution had been in contact with the plant roots for 24 hours it was more alkaline, 6.2 to 6.4, but about the same in all series. The iron content of the initial plants or a trace of iron in the salts employed was sufficient for subsequent growth. Ferrous sulphate, however, was applied to some of the cultures in certain experimental trials described on page 617. Conditions of temperature and humidity during the progress of the experiments were suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 23° C.

Chemical methods

For macrochemical analyses, the plants were divided into seven fractions as shown in table VI. All terms are self-explanatory, except the term petioles, which in this paper includes also the rachis and the large veins of the leaf. Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissues respectively, according to procedure previously described in detail (41). Aliquots of dried tissue were employed for mineral analyses (3).

Microchemical tests, which were made on fresh plant material, were in general those recommended by ECKERSON (12, 16), with modifications that will be described along with the presentation of results.

Results

On June 17, at the start of the various nutrient treatments, all of the plants were somewhat stiff and woody, with yellowish green leaves and a few blossoms. Macrochemical analysis showed that they contained no nitrates and no ammonia. They were very low in all forms of elaborated nitrogen but high in carbohydrates (tables II, III, IV, and V). Micro-

scopic examination of the stem showed a high percentage of very thick-walled mechanical and conductive tissue. Even though the plants were growing very slowly, cambium was active throughout the length of the stem. Starch was observed in large quantities in all parenchymatous tissue, especially in the pith, almost to the tip of the stem.

The initial plants contained the following percentage of calcium computed on a green weight basis; stems 0.13, blades 0.32, petioles 0.10, and roots 0.05. Calcium was present in three different forms: (1) calcium oxalate crystals, in the parenchyma of phloem, cortex, and pith; (2) "uncombined" calcium, *i.e.*, calcium that could be detected microchemically by the usual treatment with oxalic acid, and (3) "combined" calicum, *i.e.* calcium that could be detected microchemically only after treatment of fresh sections with a strong base such as NaOH. The terms "combined" and "uncombined" are purely relative, for, of course, all calcium in a cell is to some extent combined. "Combined" calcium was found in all living cells, not only along the walls but rather uniformly distributed through the protoplasts. "Uncombined" calcium was found throughout the protoplasts of all living cells. In collenchyma and parenchyma cells of the cortex and in parenchyma cells of the vascular cylinder, it occurred also along the walls, suggesting the presence of calcium pectate in the middle lamella. Many of the cells, however, especially of the pith, did not show a reaction which would indicate a middle lamella composed of calcium pectate in either plus- or minus-calcium plants.

PLANTS WHICH RECEIVED THE COMPLETE NUTRIENT SOLUTION

Throughout the course of the experiments (June 17–July 9) the plants that received the complete nutrient treatment exhibited a vigorous and

FIG. 1. Tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

apparently healthy growth of tops and roots, as shown in figures 1 and 2. Although these plants received a solution fairly high in concentration of nitrates (table 1), they were not soft nor extremely succulent. This was undoubtedly associated with the fact that the initial plants were hard,
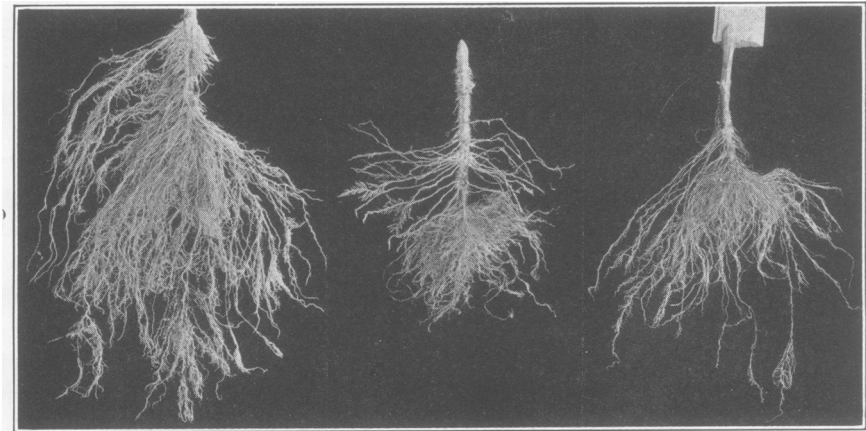

FIG. 2. Roots of tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

high in carbohydrates and low in nitrogen (tables II, III, IV, and V), also that the experiments were run for a period of only three weeks. Dur-

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS. (PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED)*

SOLUTION	$Ca(NO_3)_2$	KNO_3	KH_2PO_4	$MgSO_4$	$CaCl_2$
Plus-Ca plus-NO₃	0.0180	....	0.0090	0.0090	....
Minus-Ca plus-NO₃	....	0.0260	0.0090	0.0090	....
Minus-Ca minus-NO₃	....	....	0.0090	0.0090	....
Plus-Ca minus-NO₃	....	....	0.0090	0.0090	0.0180

* With the exception of the minus-Ca minus-NO₃ solution, each solution has a total osmotic concentration value of 2 atmospheres.

ing that time many fruits were set, and if the plants had been grown for a sufficient number of weeks undoubtedly they would have borne a heavy crop of fruit, as did the few plants which were allowed to remain.

During the progress of the experiments, certain factors remained practically constant in the various series of plants. The pH of the expressed sap of the various plant fractions, such as roots, lower stem, upper stem, stem tip, and blades showed little variation from one series to

another, although the usual (20) differences in pH of different plant fractions were observed. Microchemical tests failed to show any consistent outstanding variation in pH of respective tissues, although the usual differences (13, 41) were noted in pH of pith, xylem, phloem and cortex.

PLANTS DEFICIENT IN CALCIUM

In contrast to the plants that had received complete nutrient treatment, those that were grown from June 17 to July 9 in a solution lacking calcium (figs. 1 and 2) were stunted in vegetative growth and the stems were stiff and woody. Increase in volume occurred for only a brief period after the transfer from soil to sand on June 17. A few blossoms were formed but practically no fruit was set. The upper leaves were characteristically yellow, not yellowish green, whereas the lower leaves, which were thick and stiff, were still fairly dark green. Shortly after July 9 the stem tips of the calcium-deficient plants died.
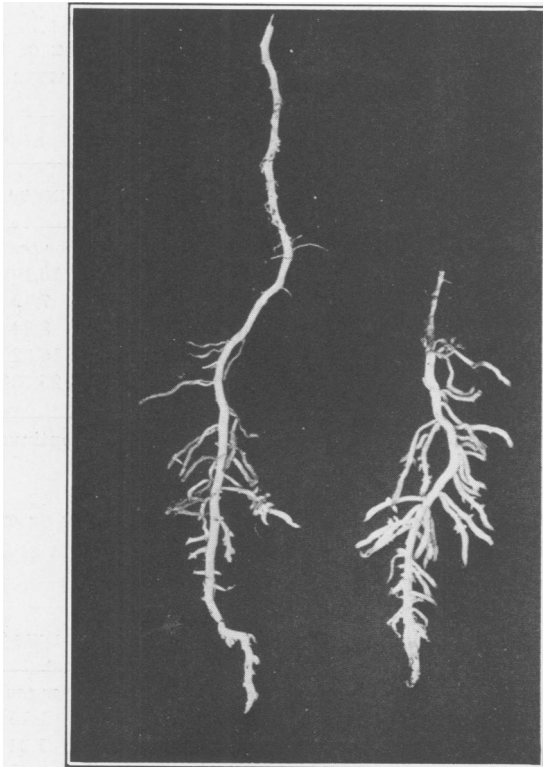

FIG. 3. Typical roots from tomato plants grown with the minus-calcium plus-nitrate nutrient treatment.

The leaves of some of the plants low in calcium exhibited a peppery brown spotting, apparently the result of iron toxicity rather than any direct effect of calcium deficiency or, on the other hand, of magnesium toxicity (page 618).

The root systems were short and much branched as shown in figures 2 and 3. Branch roots were short and stubby, and some showed characteristic browning of tips and of parenchyma further back, and sloughing off of some of the outermost cells. During the period of the experiments, however, the roots were alive and developed an abundance of apparently healthy root hairs.

The characteristics just noted were substantiated by macro-analysis and microchemical tests. Tables II and III show that the minus-calcium plants were even higher in carbohydrates than the plants that had received the complete nutrient treatment. Associated with the higher concentration of carbohydrates were thicker cell walls in the recently formed xylem.

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Dry matter	14.35	12.25	14.30	13.55	16.10	12.66
Reducing sugars	7.33	2.36	6.27	3.70	7.19	6.40
Sucrose	8.91	5.25	7.96	6.21	8.33	3.99
Total sugars	16.24	7.61	14.23	9.91	15.52	10.39
Starch and dextrin	23.74	8.92	19.17	10.04	25.73	16.54

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE III

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Reducing sugars	1.05	0.29	0.90	0.50	1.15	0.81
Sucrose	1.28	0.64	1.14	0.84	1.34	0.51
Total sugars	2.33	0.93	2.04	1.34	2.49	1.32
Starch and dextrin	3.41	1.09	2.74	1.36	4.14	2.09

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

Nitrates were comparatively low in the minus-calcium series, but on the basis of macrochemical determinations there was no significant difference between the calcium-deficient and the complete-nutrient plants in percentage or quality of organic nitrogen (tables IV and V). The quality of protein, however, was undoubtedly very different in the stems of the two lots of plants, as in the cells of the plants of the minus-calcium series,

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Total nitrate-free N...	0.553	1.530	1.375	1.609	1.649	1.570
Protein N	0.400	0.930	0.975	0.993	1.019	0.997
Nitrate-free soluble N	0.153	0.600	0.400	0.616	0.630	0.573
Basic N	0.020	0.045	0.044	0.119	0.128	0.130
Amino N	0.119	0.462	0.300	0.385	0.370	0.273
Amide N	trace	0.068	0.048	0.127	0.104	0.125
Ammonia N	none	trace	trace	0.002	0.002	0.003
Other N	0.014	0.025	0.008	− 0.017	0.026	0.042
Nitrate N	none	0.040	0.025	0.211	0.086	0.270
Total N	0.553	1.570	1.400	1.820	1.735	1.840

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE V

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Total nitrate-free N...	0.079	0.187	0.197	0.218	0.265	0.199
Protein N	0.057	0.114	0.139	0.135	0.164	0.126
Nitrate-free soluble N	0.022	0.073	0.058	0.083	0.101	0.073
Basic N	0.003	0.006	0.006	0.016	0.021	0.016
Amino N	0.017	0.057	0.043	0.052	0.059	0.035
Amide N	trace	0.008	0.007	0.017	0.017	0.016
Ammonia N	none	trace	trace	trace	trace	trace +
Other N	0.002	0.002	0.002	− 0.002	0.004	0.005
Nitrate N	none	0.005	0.003	0.028	0.014	0.034
Total N	0.079	0.192	0.200	0.246	0.279	0.233

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

particularly in the phloem region, but also in other parenchymatous tissue, there were granular inclusions that were not evident in the complete-nutrient plants. These inclusions gave positive protein reactions with all the usual reagents. Furthermore, such inclusions were conspicuous in the stem tips of calcium-deficient plants at the time of death of the meristem and shortly before.

Internally the stubby bulbous roots of the plants grown in the solution deficient in calcium showed differentiation of stele practically down to the embryonic tip, whereas in the faster growing roots of the complete-nutrient plants the rapid division and elongation of cells resulted in a long region of elongation between embryonic tip and zone of maturation. Within the stubby tips the planes of cell division were not irregular, although enlargement of cells was greater laterally than longitudinally. A factor which also contributed to the general appearance of stubbiness of the root system was the development of primordia of numerous branch roots, many of which never developed. Constrictions of the roots were not uncommon and were due to localized injury of outermost layers of cells and failure of these cells to enlarge properly, or to disintegration, or to both.

The externally conspicuous browning of roots of the calcium-deficient plants was almost entirely of parenchymatous and meristematic tissue. This was almost always observable first in the root tip, in the endodermis and in isolated cells of the cortex and stele. Later it occurred in practically all parenchymatous tissue, but did not appear to be present in conductive elements of xylem and phloem. Cellulose walls were chiefly concerned, but the protoplasts sometimes became decidedly granular, and this granular mass, as in the stems, was often a golden brown color and gave positive tests for protein with the usual reagents; the reaction with Millon's reagent was particularly strong.

The plants were tested for calcium from time to time during the course of the experiments. Microscopic examination showed the presence of some calcium oxalate in the base of the stem even at the end of the experiment, long after the plants had begun to show signs of calcium deficiency. The usual microchemical test with oxalic acid gave practically negative results, showing that there was little "uncombined" calcium present. However, an appreciably greater quantity was detected by treatment with oxalic acid after the sections had been treated with NaOH. These results suggest that the considerable quantity of calcium shown by macro-analysis to be present in the lower half of the tops (tables VI and VII), consisted largely of calcium oxalate deposits and of calcium so combined with protein or some other substance that it had to be released by hydrolysis before it could react with oxalic acid.

TABLE VI

MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF DRY MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
SERIES	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Upper blades	1.61	0.17	0.67	0.60	0.80	0.49	2.67	3.65	0.98	0.59
Lower blades	3.84	1.10	0.99	0.85	0.50	0.31	2.67	3.28	1.45	1.01
Upper petioles	1.08	0.11	0.92	0.23	0.73	0.78	6.27	9.64	0.38	0.51
Lower petioles	2.23	0.26	1.64	0.33	0.69	0.51	4.70	10.62	0.49	0.49
Upper stem	0.67	trace	0.69	0.24	0.70	0.68	5.25	6.02	0.33	0.31
Lower stem	0.99	0.53	0.50	0.30	0.41	0.43	2.27	4.39	0.28	0.25
Roots	1.26	0.25	0.46	0.60	2.34	0.72	2.23	3.18	0.75	0.55

TABLE VII

MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF GREEN MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
SERIES	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Upper blades	0.255	0.028	0.106	0.097	0.127	0.079	0.425	0.592	0.156	0.096
Lower blades	0.595	0.254	0.153	0.197	0.078	0.072	0.414	0.759	0.225	0.234
Upper petioles	0.935	0.010	0.080	0.021	0.063	0.074	0.543	0.909	0.033	0.048
Lower petioles	0.245	0.029	0.180	0.034	0.076	0.052	0.517	1.062	0.054	0.051
Upper stem	0.057	trace	0.059	0.030	0.060	0.085	0.452	0.759	0.028	0.042
Lower stem	0.150	0.087	0.076	0.050	0.062	0.071	0.345	0.724	0.043	0.041
Roots	0.149	0.035	0.054	0.084	0.276	0.101	0.263	0.447	0.089	0.077

PLANTS DEFICIENT IN CALCIUM AND NITRATES

The plants that throughout the experiments received a solution lacking in both calcium and nitrates, were in some respects like those that lacked calcium only. They too increased only slightly in size (even less than the minus-calcium series) and were woody in texture; microchemical tests showed that they too were high in starch and devoid of nitrates. Also, they contained practically no uncombined calcium but did contain a considerable quantity of combined calcium. However, they were not so yellow (fig. 1), and what yellowing occurred was chiefly of the older leaves, and not of the tips as observed above in the minus-calcium series. Moreover, the root systems were without the stubbiness of branch roots noted in the plants that lacked calcium only (fig. 3). This difference in appearance of roots and tops may have been due to the fact that with less vegetative extension there was a higher concentration of calcium per unit volume of plant tissue. It will be recalled that the vegetative growth of the plants of the calcium deficient series occurred for only a brief period after the start of the experiments. At that time all plants contained an abundance of calcium (page 607), were, in effect, plus-calcium plants, and were undoubtedly able to absorb and assimilate nitrates if present in the nutrient solution. On the other hand, in the series deprived of nitrates, no nitrates were available at the time of the initial growth of the other series of plants, when calcium was still available.

In an effort to study the absorption of nitrates, some of these plants deprived of both calcium and nitrates were shifted to the nutrient solution containing nitrates but lacking calcium. Hourly examination of these plants for nitrates, day and night for a period of five days after supplying nitrates, showed that during that period nitrates were not absorbed. Only after seven days was a minute trace of nitrates found in the roots, and later a little in the tops of the plants.

The fact that Dr. ECKERSON found practically no reducase in the roots of these plants nor in the roots of any of the calcium-deficient plants would seem to preclude the possibility of absorption and simultaneous complete assimilation of nitrates. Further, no nitrates and practically no ammonia were found in these plants at any time. In connection with reducase activity attention may be called to the relatively low phosphorus content of the roots of the calcium-deficient plants (tables VI and VII).

Although these plants of the minus-calcium minus-nitrate series did not absorb nitrates, they absorbed calcium instantly when shifted to the complete nutrient treatment. In forty-five minutes the entire root system contained uncombined calcium, and half an hour later there was a considerable quantity of calcium throughout the tops. It was, however, twelve

hours after the shift to plus-calcium treatment before these plants began to absorb nitrates from the solution, and about sixteen hours before faint traces of nitrites were observed.

DARKNESS TREATMENT

An attempt was made to study the hydrolysis and re-utilization of substances in darkness. On July 1, some of the plants that had been grown in the solution deficient in calcium only, were shifted from the greenhouse to a dark room that was maintained at about 23° C. The plants were kept in continuous darkness until July 9. During that period the stems elongated several inches, and the previously formed upper leaves turned from yellow to green, although newly formed leaves were yellow. As is indicated in tables IV and V there was an increase in concentration of nitrates. Further, microchemical tests showed that after the darkness treatment there was considerable uncombined calcium present, whereas other plants of the same series in the light contained practically no uncombined calcium.

It is apparent from tables II and III that carbohydrates decreased greatly during the period of darkness, yet there was much starch even in the tissues of the stem and leaves that were newly formed in darkness. The gross macrochemical analyses of stems (tables IV and V) show little change in percentage of protein or other elaborated nitrogen. There must have been, however, very definite changes in these substances, otherwise the rapid growth of meristem of the stem and the elongation of cells could not have occurred. Unfortunately macro-methods for proteins do not differentiate between those of the meristem and those of mature tissue. Microchemical tests showed, however, that during the period in darkness the granular masses of protein material (page 612) practically disappeared and much meristematic tissue of stems and roots developed. In these regions there were also observed appreciable quantities of asparagine.

On July 1, some of the plants that had been grown with no external supply of either calcium or nitrates were also placed in darkness. The same growth responses occurred. With the exception that nitrates were absent, similar chemical changes also took place, including the accumulation in the plants of considerable uncombined calcium that was not present in plants of the same series in the light.

After the darkness treatment, the plants (minus-calcium-minus-nitrate) were given a solution containing nitrates, but as before no calcium. Nitrates were observed in the small roots within fifteen minutes and throughout the plant within one hour. Such a rapid intake of nitrates was in striking contrast to the lack of absorption by plants of the same series in the light.

The fourth series of plants, those that were grown with no external supply of nitrates but with an abundance of calcium and other essential elements (plus-calcium-minus-nitrate solution, table I) were typical nitrogen-deficient plants (**13, 27, 40**). All the leaves, but especially the lower ones, were yellowish green with purple veins. The stems were hard and woody, very high in starch and free of nitrates.

These plants when shifted to minus-calcium-plus-nitrate treatment absorbed nitrates instantly. In less than an hour nitrates were present in all parts of the plant, and as usual (**13, 40, 41**) nitrites were observed in large quantities for three or four hours following the initial absorption of nitrates.

At the time of shift in nutrient treatments, the plants were observed for calcium distribution. Parenchymatous cells that were filled with masses of calcium oxalate crystals were conspicuous in phloem, cortex and pith. They were present in large numbers even in the extreme tip of the stem, and in parenchymatous cells of all parts of the root system. After transfer from the plus-calcium-minus-nitrate to the minus-calcium-plus-nitrate solution there was within a few days and for only a short time renewed vegetative growth. The concentration of uncombined calcium remained practically constant for about ten days, although there was a steady decrease in combined calcium during the same period. At the end of ten days, masses of calcium oxalate crystals were conspicuous in the base and middle of the stem, but none were present within five, and very few within ten, centimeters of the tip. The disappearance of calcium oxalate masses seemed to be first from the phloem and later from the cortex and pith. At the end of four weeks the concentration of combined calcium was very low, there was practically no uncombined calcium, and the stem tips were practically dead. Yet at that time calcium oxalate deposits were observed in the base of the stem and to a much lesser degree in the lower petioles. In many instances the masses looked as if they were undergoing decomposition.

Thus the re-utilization of calcium oxalate proceeded slowly and incompletely from the tip backward, and the plants died while crystals of calcium oxalate were still present in the base of the stem.

Discussion

If seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen, phosphorus, or potassium, the effects of nitrogen- or phosphorus-deficiency are evident in a comparatively short time (**13, 40, 15**), but conspicuous symptoms of lack of potassium (**41**) are not usually

apparent until much later. Although the symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of tomato plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost leaves and tip of the stem are fairly dark green and may remain so for a considerable period.

On the other hand, the appearance of the calcium-deficient plants of these experiments was very distinctive, unlike tomato plants deficient in nitrogen, phosphorus, or potassium, in that the upper part of the plant was yellow rather than green, and the lower half instead of being yellowish with practically dead leaves was fairly dark green.

Closely following the lack of chlorophyll in the tops, a condition which has been observed even in the lower plants (5, 17, 45, 46), there was a cessation of meristematic activity and death of tissue. This may, of course, occur when any essential element is deficient but it occurred in the tomato plants of these experiments within three weeks of minus-calcium treatment. Similar observations on tobacco plants were made by GARNER (17), who found that a lack of calcium affected most seriously the upper leaves and embryonic tissues of the growing point. He found that deficiency of magnesium, like deficiency of nitrogen, phosphorus or potassium, affected particularly the lower half of the plant.

The peppery brown spotting observed in the leaves of some of the calcium-deficient plants was probably iron toxicity, and was not due directly to insufficient calcium. Certainly it is not a distinguishing characteristic, although others (17, 43) have reported that these symptoms occurred when plants were grown in a deficiency of calcium. Analyses of leaf blades of the several series showed that there was often somewhat more filterable iron in the minus- than in the plus-calcium plants. Furthermore, the peppery spotting was easily obtained at will in plants of any of the series of these experiments but particularly in the calcium-deficient plants, simply by adding ferrous sulphate to the nutrient solution at the rate of ten or twenty milligrams of iron per liter. Respective tissues of calcium-deficient plants were not, however, more acid than those of the other series (page 608). If they had been, the higher percentage of soluble iron might be explained on the basis of SHIVE's (53) results, for he has found that soluble iron is relatively high when cell sap is acid, relatively low when cell sap is more alkaline. On the other hand experimental work by SMYTH (54) has indicated that quality of acid may be an important factor in its influence upon the form of iron present, and work by GARNER (17) suggests that mineral deficiencies may directly or indirectly affect the quality of organic acids in a plant. In our experiments specific acids were not determined.

In view of popular theories (57) it also appeared possible that magnesium toxicity might be a factor in the growth responses obtained. However, when concentrations of magnesium sulphate more than three times as high as that of the regular nutrient solutions (table I) were applied for a period of two weeks to some of the plants not employed for analysis or other purposes, no spotting of the leaves nor any other visible effect upon the plants was observed in any of the series. The original total molecular concentration of the nutrient solutions was maintained by decreasing the concentration of potassium phosphate in proportion to the increase in magnesium sulphate. Likewise a few plants of the several series were grown for two weeks with no external supply of magnesium by substituting potassium sulphate for magnesium sulphate in equivalent concentration in the nutrient solution. The temporary removal of magnesium from the nutrient solution produced no visible effect on the growth responses of the plants. It is also apparent from tables VI and VII that there was no marked excess nor deficiency of magnesium in the minus- as compared with the plus-calcium plants. Further, PFEIFFER (42) shows that in oat plants the ratio of calcium to magnesium may vary within rather wide limits with no noticeable effect upon the plant.

It has been reported (10, 55) that lack of abundant calcium results in short, stubby, bulbous roots that are characteristically brown at the tips, with sloughing off of cells further back. The calcium-deficient tomato plants of these experiments were no exception. Internally there was differentiation of xylem and phloem practically down to the embryonic tip, which means, of course, that they were growing very slowly as compared with the plus-calcium roots, in which active division and elongation of cells resulted in a long region of elongation between embryonic tip and region of maturation. In the stubby tips of the calcium-deficient plants, however, distortions and irregular planes of division mentioned by SOROKIN and SOMMER (55) were not evident. The cells seemed entirely regular, although particularly in the cortex the cells enlarged somewhat more laterally than longitudinally.

The conspicuous browning of roots, a symptom very commonly associated with calcium deficiency, was due mainly to browning of meristem and parenchymatous tissue of the cortex. The stele seemed little affected and there were numerous root hairs that did not appear materially different from those of the plus-calcium plants except that in general they were somewhat shorter. The browning was mostly of the cell walls, which in healthy parenchyma are considered to be composed mainly of cellulose and pectic materials. The nature of change that resulted in browning of the cell walls was not determined. It is not uncommon, however, in tomato roots and may be produced by many types of injury.

No definite information was secured as to the presence or absence of a middle lamella of calcium pectate. After treatment with oxalic acid, rows of small calcium oxalate crystals were frequently observed along cell walls in the cortical tissue of roots of the plus-calcium plants, possibly indicating the presence of a middle lamella of calcium pectate. Although such a reaction was not invariably obtained in all cells even in the plants grown with an abundance of calcium, yet on the other hand, it was not observed at all in roots of the calcium-deficient plants that showed much browning and sloughing of outer cortical cells. It has, however, been reported by others (**36, 45, 61**) that in a deficiency of calcium a middle lamella of calcium pectate may not develop.

Whereas the browning of roots was mainly in cell walls, the protoplasts of the meristem and older tissue also became decidedly granular, and this mass was usually golden brown in color and gave strong protein reactions. Apparently in a deficiency of calcium, as in a lack of phosphorus (**15**) or potassium (**41**), there is definite interference with formation of proteins essential to the protoplasts of active cells.

Many different workers (**4, 19, 22, 52**), using various kinds of plants have found that carbohydrates frequently accumulate in the tissues of plants deficient in calcium. It has been shown, however, (page 617) that calcium limitation directly or indirectly interferes with chlorophyll formation. Therefore, under certain experimental conditions and in a deficiency of calcium the formation of chlorophyll might be so limited as to seriously decrease the rate of assimilation of carbon dioxide and thereby prevent accumulation of carbohydrates. This was possibly the case in results reported by BURRELL (**6**). Nevertheless, calcium deficiency seems quite commonly to result in carbohydrate accumulation, and the enormous concentration of sugars and starch in the low calcium plants of these experiments is clearly shown in tables II and III. Microchemical reactions also showed that starch was present in great abundance throughout all parenchymatous tissues, even in the tips of roots and stems.

In explanation of such observations it is usually said that calcium is essential for digestion of starch and translocation of sugars, and that in a lack of this element carbohydrates therefore accumulate. But it is evident that there is no sound basis for this notion, because in the calcium-deficient plants of these experiments sugar and even starch grains were found in the most distal portions of roots, stems and leaves. In what manner translocation of carbohydrates might be more complete is indeed a question. Also SCHIMPER (**52**) has shown that digestion of starch and translocation of sugars take place in leaves and stems of plants that contain a very low concentration of calcium. Further, the minus-calcium tomato plants of

these experiments after a few days in darkness decreased greatly in car-
bohydrates (tables II and III). Likewise, microchemical reactions showed
the presence of an abundance of sugar and starch in tissues of stem and
leaves that were yellow in color and newly formed in darkness. Thus there
is additional evidence showing that there may be digestion of starch,
translocation of sugars and re-condensation of sugars to starch in plants
extremely low in calcium (tables II and III). It should be pointed out,
however, that during the period in darkness there was an increase in
uncombined calcium (page 615), although it does not appear probable th⁻ᵗ
this was a direct factor in effecting digestion of starch and translocation of
sugars, for the latter process began to occur considerably in advance of
any apparent change in form of calcium.

One of the principal uses of carbohydrates is in protein synthesis. It
has been shown repeatedly (**13, 27, 39, 40**) that carbohydrates may accumu-
late if a plant has no external supply of available nitrogenous nutrient.
They may even accumulate with nitrates present in abundance in ᵗ'
nutrient medium or even in the tissue of the plant, if there is little reducase
activity due to a deficiency of potassium (**41**) or phosphorus (**15, 28**).
Both elements are necessary for synthesis of nitrates to protein.

When the plants were shifted from soil to sand culture at the beginning
of these experiments all were in effect plus-calcium plants. At that time
and for a short period thereafter, there was presumably some protein
synthesis by the plants that were receiving minus-calcium nutrient treat-
ment. However, carbohydrates undoubtedly accumulated in these plants
because the amount of protein synthesis was small.

There are two reasons for the small amount of protein synthesis. First,
the plants were very much limited in their ability to absorb nitrates (pₐ ⁻ᵒ
611), and second, the small quantity of nitrates absorbed (tables IV and
V) remained for the most part unelaborated, as Dr. ECKERSON found that
the calcium-deficient plants, especially the roots, were very low in reducase
(nitrate reducing material). Apparently there was little synthesis of even
the simpler organic compounds of nitrogen. The minus-calcium plants
increased very little in volume. Accordingly, if there had been synthesis
of simpler forms of organic nitrogen or protein it would be evident on a
percentage-of-green-weight basis; but table V indicates that this did not
occur. The effects of phosphorus (**15, 28**) or potassium (**41**) deficiency
are somewhat different.

The concentration and quality of the various nitrogenous fractions of
the whole stems were about the same in plus- as in minus-calcium plants
(tables IV and V), yet microchemical reactions and anatomical examination
showed decided differences. There was comparatively little meristematic

tissue in roots or stems of the calcium-deficient plants, but there were present in the region of phloem and inner cortex of roots and stems, cells which contained golden brown granular masses of material that was in large part proteinaceous. These protein-like inclusions may not be peculiar to calcium deficiency, as a somewhat similar condition may occur in a lack of phosphorus (15) or potassium (41), but apparently the very early death of embryonic tissue is a factor invariably accompanying a lack of calcium (10, 17, 52, 55). This element appears to be directly or indirectly necessary for development of proteins essential to the protoplasts of active cells. Without calcium, proteinaceous inclusions accumulate, not only in tomato but in other plants (58).

It is not apparent why a deficiency of calcium almost completely prevented absorption of nitrates (page 614), when the same plants that failed to absorb nitrates took in calcium instantly. The experimental results fail to indicate whether or not calcium deficiency limits the absorption of materials other than nitrates. Attention may, however, be called to tables VI and VII. The concentration of sulphur is much the same in both plus- and minus-calcium plants. Both lots of plants also contain about the same percentage of magnesium with the exception of the petioles of the calcium-deficient plants, which are comparatively low in this element. The concentration of potassium is comparatively high in the plants lacking calcium. This is a condition which has often been observed by others (31, 32, 47). It should be remembered, however, that the minus-calcium nutrient solution (table 1) was comparatively high in potassium.

There seems to be nothing in the literature which has any very significant bearing on the inability of the minus-calcium plants to absorb nitrates,[2] although it is well known (18, 29, 30) that leguminous plants especially are high in nitrogen if the soil is abundantly supplied with calcium. The root systems of the low-calcium tomato plants were, of course, abnormal (pages 610 and 614) yet other essential elements were absorbed. The usual theories (57) on permeability are, in fact, opposed to the results obtained. Also, work by TRUE (61) and ECKERSON has shown that certain materials may be absorbed more readily by plants

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[2] It seems impossible to draw conclusions from the work of JERMAKOW (23). He placed excised leaves in plus- and minus-calcium solutions and analyzed the leaf tissue for nitrates. At least as far as absorption is concerned, excised leaves can scarcely be compared with an intact root system. He found, however, a greater concentration of nitrates in tissues of the minus-calcium leaves. He assumes, therefore, that there was more rapid assimilation (disappearance) of nitrates in the leaves which received an external supply of calcium. He did not, however, analyze his residual nutrient solutions, nor did he determine other nitrogenous fractions. He has therefore no measure of nitrates absorbed. Further, his leaves were presumably obtained from soil-grown plants, and it is doubtful if they were deficient in calcium.

deficient in calcium than by others in a full nutrient solution. These workers did not, however, determine the ability of a calcium-deficient plant to absorb nitrates.

Whether or not the low phosphorus content of the roots of the minus-calcium plants was a factor affecting nitrate absorption is not apparent. Only total phosphorus was determined.[3] Although phosphatides are said to be important in determining the permeability of wall or protoplast (57) the question is at best unsettled (56), and little can be gained by discussion. It may, however, be pointed out that CHIBNALL (8) reports the presence of a calcium phosphatidate in cabbage.

In connection with the low content of total phosphorus in the calcium-deficient roots, it may be emphasized that there rather than in the tops reducase was lowest. Also, ECKERSON's work (15) indicates that a lack of phosphorus limits reducase activity apparently more than a deficiency of other essential elements.

A series of excellent experiments by THERON (59) showed that the removal of nitrates was less from an alkaline than from an acid solution. Likewise SABININ and KOLOTORA (51) obtained somewhat similar results. However, in recent work (50, 60) with the tomato the pH of the nutrient medium did not materially influence absorption until the plant became apparently saturated with nitrates. In an alkaline medium, nitrate saturation within the plant occurred in a very short time; thereafter the plants did not absorb nitrates. The plants grown at a high pH had the ability to *absorb* nitrates but were unable to synthesize them to amino acids or protein.

The literature (9, 11, 37, 59, 62) is full of contradictory statements as to the effect of calcium deficiency on the pH of plants. Where whole tops are employed as a sample, the pH is usually lower in the calcium-deficient plants than in the more vigorously growing plants that have had an adequate supply of calcium; the difference can be accounted for by the fact that there is a larger amount of meristematic tissue, notably alkaline (20), in the latter. However, in homologous fractions of plus- and minus-calcium tomato plants the tissue and expressed sap were not materially different. The pH of nutrient solutions was also essentially the same in each of the respective series, practically optimum for maximum absorption and assimilation of nitrates (50, 60). Accordingly it does not seem probable that pH of plant or of nutrient solution was a limiting factor in the inability of the minus-calcium plants to absorb nitrates.

The failure of the calcium-deficient plants to absorb nitrates does not appear possible of explanation at this time. It is significant, however, that there was very rapid absorption of nitrates in low-calcium plants that had been subjected to a period of darkness (page 615). Within the minus-

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[3] Microchemical tests for calcium phosphate gave negative results.

calcium plants conspicuous changes were associated with darkness treatment; these included proteolysis involving the loss of many of the granular proteinaceous inclusions, decrease in combined calcium, and increase in uncombined calcium. RISSMAN (49) also records a decrease in concentration of insoluble calcium for wheat grown in darkness, and RAMANN's (44) results indicate that there is more mobile calcium at night than during the day. The chemical significance of this change in form of calcium is not known. It is obvious that uncombined calcium alone (at least when newly absorbed) was not sufficient to result directly and immediately in nitrate absorption, for much uncombined calcium was present throughout the minus-calcium plants one hour after the shift to plus-calcium treatment. Nitrates, however, as determined by their complete absence and by lack of reduction products, were not absorbed until twelve hours later.

Apparently before nitrate absorption could take place other changes were necessary, changes probably involving the combination of calcium with protein or other materials. It is doubtful if proteolytic changes were a direct factor in either case, as only twelve hours of plus-calcium treatment were required before nitrates were absorbed. During such a short period of time there could scarcely have been extensive synthetic or proteolytic changes. But proteolysis or other catabolic changes in darkness released combined calcium. In another part of the experiment uncombined calcium was furnished by an external supply of soluble calcium salts. In both cases there apparently followed a re-combination with newly formed proteins or other materials. A supply of amino acids for formation of new protein was available in both instances; in the minus-calcium plants in darkness, through protein decomposition to amino acids, and in the plants newly supplied with calcium, through nitrate reduction to amino acids (page 615).

Non-absorption of nitrates was not a direct effect of a poorly proportioned nutrient solution, as the series of plants containing calcium but lacking nitrates (plus-calcium minus-nitrate, table I) absorbed nitrates instantly from the minus-calcium plus-nitrate solution.

Neither was darkness a direct factor in effecting nitrate absorption by the calcium-deficient plants. Moderate shading produced the same results but in lesser degree. Undoubtedly cloudy weather would tend to have the same effect, and probably the short days of winter in case of tomato (38, 41). All these conditions are apparently essentially similar in that they result in proteolysis, in a decrease in carbohydrates, and in the release of combined calcium for recombination with material of the protoplast. Likewise, if the initial plants of these experiments had been very low in carbohydrates and high in the simpler forms of organic nitrogen (40), a greater degree of re-utilization, or less combination of calcium might have taken

place and different results might have been obtained with respect to nitrate absorption. This point is to be investigated. However, a low concentration of organic nitrogen accompanied with a high percentage of carbohydrates in the plant does not in itself inhibit nitrate absorption. The plants of these experiments that were high in calcium and deficient in nitrates were low in all forms of nitrogen and extremely high in carbohydrates, yet they absorbed nitrate in abundance. But they also contained an abundance of uncombined calcium, which was available for combination with materials of newly formed cells. Also they were very high in reducase activity, as is usual for plants of such quality.

There appears to be but fragmentary evidence as to the forms of calcium found in plants, and much of this is misleading on account of the fact that air dried tissue has been employed for analysis (**2, 25, 49**). Kostytschew and Berg (**25**) report that calcium does not occur in organic combination with protein. Their results, however, do not warrant this conclusion.[4] On the other hand, there is apparently no proof that calcium combines with proteins, although in the body fluids of animals it is said (**35**) to form a non-ionized protein compound. It would, however, appear significant that increase in uncombined calcium closely paralleled changes in the minus-calcium tomato plants which must have involved proteolysis (page 615). There may, of course, have been significant changes in other materials such as possibly phosphatidates of calcium. There is, however, increasing evidence tending to indicate that mineral elements may exist in combination with protein (**24, 33, 35, 54, 63**).

There are available many determinations (**21, 48**) of total water-soluble calcium. Such results, however, tell nothing as to the actual calcium compounds present. In many cases calcium oxalate undoubtedly accounts for much of the insoluble calcium fraction, and probably calcium phosphate under some conditions. Also much of the insoluble calcium is present as *combined calcium* (page 607). The proportion of soluble and insoluble calcium varies (**21, 48**), yet there seems always to be considerable insoluble calcium, whereas potassium is practically all soluble and readily translocated from mature to embryonic regions (**41**).

In calcium-deficient plants, however, it has been observed that most of the calcium present is in the older tissue of roots and tops (**17, 21, 46, 61**). Likewise, the low-calcium tomato plants (tables VI and VII) contained con-

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[4] Kostytschew and Berg first dried plant tissue, apparently at room temperature, and extracted with cold water. Yet it has been shown by Chibnall (7) and others that such treatment results in extensive proteolytic changes; changes which in the tomato at least are associated with liberation of combined calcium. Following aqueous extraction these workers treated the tissue with 2N acetic acid and 2N hydrochloric acid. As might be anticipated, the ash of the residue was calcium-free, as either of the reagents employed react with the usually recognized calcium constituents of the plant.

siderable calcium in the lower half of the stem, yet there was only a trace in the upper stem. In this connection may be recalled the external appearance of the calcium-deficient plants, yellow in the upper half of the tops but green below. In contrast, in plants that are deficient in phosphorus (**15**), nitrogen (**14, 27, 40**), or potassium (**41**), the lower leaves are yellowish but the tips are comparatively green, and the greatest concentration of these elements is found in the tips or in other embryonic tissue (**15, 27, 40, 41**). Further, practically all the calcium present in fresh tissue of the minus-calcium tomato plants was found to be water-insoluble. Some of it was present as calcium oxalate deposits in mature tissues, but much of the insoluble calcium was in another form (combined calcium) that reacted with oxalic acid only after treating sections with alkali, or after the plants had been subjected for several days to continuous darkness or shading.

It has been shown by various workers (**26, 34**) that there may be to some extent re-utilization of calcium oxalate. The low-calcium tomato plants utilized calcium oxalate (page 616), but the crystals were dissolved by the plant so slowly that the embryonic tissue of stem and root tip died while there were yet heavy deposits of calcium oxalate in the base of the stem and in the lower petioles.

In addition to calcium oxalate crystals, there was combined calcium in older tissue of low-calcium plants when they died. Stimultaneously, however, with disappearance of uncombined calcium there was death of meristematic tissue. When new cells are formed there must be not only calcium presumably for the middle lamella, but available calcium for combination with materials of the protoplast. It seems quite probable that some of this material may be protein, as without calcium inclusions accumulate in the protoplast in the form of granular masses that are, at least in large part, constituted of proteins (page 612).

Summary

1. The upper parts of the tops of calcium-deficient tomato plants were yellow and the lower leaves and stems were fairly green. (In a deficiency of nitrogen, phosphorus or potassium respectively, the lower portion of the plant is yellowish, but the upper leaves and stem remain green.)

2. The roots were characteristically short, bulbous and brown at the tips, with sloughing off of cells further back. The roots were short because of slow growth of the meristem, and bulbous because cortical cells enlarged somewhat more laterally than longitudinally. The failure of lateral root primordia to develop also contributed to the bulbous appearance.

3. Sloughing off of cells was probably due in part to the fact that a middle lamella of calcium pectate did not develop in roots of tomato plants extremely deficient in calcium.

4. Browning of the roots occurred in the cell wall and in the protoplast. The latter was composed in part of granular proteinaceous material. Such protoplasts were also present in the stem, especially near the meristem.

5. Calcium-deficient tomato plants of these experiments under the seasonal light conditions of the greenhouse were practically unable to absorb or assimilate nitrates although they absorbed calcium instantly.

6. The plants which lacked calcium accumulated carbohydrates in large quantities, apparently because absorption and assimilation of nitrates did not take place.

7. Translocation of sugars and digestion of starch took place freely in tomato plants extremely low in calcium.

8. Nearly 100 per cent. of the calcium of fresh tissue of the calcium-deficient plants was water-insoluble and most of it was located in older tissues of roots and tops. (In a deficiency of nitrogen, phosphorus, or potassium respectively, the highest concentration of the deficient elements is in young embryonic tissues.)

9. Some of this insoluble calcium was present as calcium oxalate, but much was in another form, *combined calcium,* that reacted with oxalic acid only after treatment with alkali.

10. In calcium-deficient tomato plants utilization of calcium oxalate and re-utilization of *combined calcium* took place, but so slowly that root and stem tips died while there were yet heavy deposits of calcium oxalate and a high concentration of *combined calcium* in older tissues.

11. When new cells are formed there must be calcium not only presumably for the middle lamella, but also available calcium for combination with materials of the protoplast. Otherwise granular proteinaceous inclusions accumulate.

12. Calcium-deficient tomato plants that were shaded or placed in continuous darkness for several days decreased in carbohydrates. Associated with the decrease in carbohydrates there was proteolysis and a noticeable increase in *uncombined calcium* and a diminished concentration of *combined calcium.*

13. Accompanying proteolysis and increase in *uncombined calcium* there was rapid formation of new stem tissue and absorption of nitrates, even though there was no external supply of calcium available.

14. Calcium-deficient plants in the light were given an external supply of soluble calcium salts. A few hours after absorption of calcium there was absorption and assimilation of nitrates, and subsequently growth was resumed.

15. Darkness 'treatment of calcium-deficient plants, and shift from minus- to plus-calcium nutrient, are apparently in certain respects similar in principle and effect. In both cases there is made available *uncombined calcium* for combination with newly formed proteins or other materials. A supply of amino acids for formation of new proteins of meristems is like-

wise available in both instances: in the calcium-deficient plants in darkness, through proteolysis, and in the plants newly supplied with calcium, through nitrate assimilation.

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