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CHLORINE, BROMINE AND SODIUM AS NUTRIENTS FOR SUGAR BEET PLANTS[1]

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Chlorine, sodium and silicon have long been considered as elements that are possibly essential to the growth and development of sugar beets and other plants. Of these elements Cl has been found just recently by Broyer et al (2) to correct a severe nutritional deficiency of the tomato plant when grown in low halide culture solutions. Raleigh (28) observed enhanced growth of table beets in culture solutions supplied with NaCl instead of Na_2SO_4 as the source of Na. However, no symptoms attributable to a Cl deficiency were reported by him. Crowther (6) noted that sugar beet plants wilted less when Na was applied as NaCl than when supplied as Na_2SO_4. Significant responses to chloride additions were observed in nutrient solutions by Lipman (21) for buckwheat, by Eaton (7) for tomatoes and cotton, and by Kretschmer et al (16) for lima bean fruit.

As for Br, it has not been considered as a nutrient favorable to plant growth, even though it has been used repeatedly in ion absorption studies by many investigators for many years.

Much has been written about Na in terms of direct and indirect effects upon plant growth (5, 9, 10, 19, 20, 23). As an indirect factor of growth the beneficial effects of Na applications to the soil have been explained frequently as a release of K from the soil, or as promoting better root development (5). These phenomena have been considered of special importance in meeting the K requirements of plants on soils low in K. Quite often too the growth of plants has been increased by the addition of Na salts to soils (4, 5, 8, 17, 36) or to nutrient solutions low in K (12, 24, 35). When plants are high in K, Na responses have been observed (12, 18, 31) but most often these are much reduced or not at all in evidence (4, 17, 18, 22, 35). Direct effects of Na on sugar beet growth in field experiments have been reported for muck soils by Harmer and Benne (9) and Harmer et al (10), for sand cultures by Tullin (32), and for table beets in pot experiments with soils by Larson and Pierre (18). Sodium deficiency symptoms have been reported (32) or described (9) in only a few instances and so far no specific function of Na, not performed by K, has been recorded (17, 19). Yet for the beet, "sodium may almost be deemed an indispensable nutrient element, approaching potassium in importance" (19).

Silicon, when added as a silicate to soils, has been considered primarily as contributing to the phosphate supply of the soil (29). Raleigh (26, 27) reported increased growth of table beets when silicates were added to culture solutions. He was unable to conclude from his work whether the favorable effect was due to a healthier root system or to a specific need for Si by the plants for growth.

In view of these reports, often indefinite in their conclusions, it was thought worthwhile to re-examine Na, Si and Cl in relation to the growth of sugar beet plants by adding these elements to otherwise complete culture solutions prepared from "chemically pure" salts. The initial experiment was of a factorial design using two levels of the elements in question. The first level of Cl, Na or Si was the amount present as an impurity in the salts and distilled water. The other level was an exact quantity added. It was thought that if the requirement for any one or more of these elements was larger than that provided by the impurities present, it would be revealed by the increased growth of the plants at the higher nutrient level. If increased growth was not obtained, the second step to be taken would include a further purification of all salts for each of the elements not found to enhance growth or development of the sugar beet plant. Two other factors were also included in the experiment; K at 2 levels and 2 beet varieties, giving a total of 5 factors each at 2 levels.

METHODS AND MATERIALS

PLANT CULTURE: The sugar beet (*Beta vulgaris*) varieties used in the 2^5 factorial experiment were U.S. 22/3 and GW 304, and for the chloride series experiment, U.S. 75. In each instance 25 gm of seed were treated with 0.25 gm of the fungicide Phygon XL (United States Rubber Company, Naugatuck Chemical Division, New York, New York). These seeds were planted 2.0 cm deep in a $31 \times 76 \times 20$ cm germinating tray filled with industrial No. 2 vermiculite (California Zonolite Company, San Francisco, California). The seeds and seedlings were watered daily with one-half strength Hoagland's culture solution (11). When the seedlings were in the early 2-leaf stage, single plants were transplanted to cork rings having approximately 6.0 cm outside and 4.0 cm inside diameters. The plants were held in place with non-absorbent cotton. Three plants prepared in this way were taken at random and inserted into a mason-

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ite cover for each culture solution tank. The tanks were standard black iron buckets of 20-l capacity that were lined on the inside with a plastic coating, Amercoat No. 33 (Amercoat Corp., South Gate, California) and on the outside with aluminum paint. All culture solutions were aerated continuously.

The compositions of the culture solutions for the 2^5 factorial experiment are given in table I. The treatments essentially involved 2 sugar beet varieties and 2 levels of Na, Cl, Si and K, giving 32 treatments in duplicate or a total of 64 separate 20-l tanks for the experiments. The plants were set out on October 12, 1953 and harvested on January 14, 1954.

The salts for the chloride series experiment were of the following initial concentrations: 0.001 M KH_2PO_4, 0.00375 M $Ca(NO_3)_2$, 0.001 M $MgSO_4$, 0.00025 M Na_2SiO_3, 0.25 ppm B, 0.25 ppm Mn, 0.025 ppm Zn, 0.01 ppm Cu, 0.005 ppm Mo. Iron was added at the rate of 2.5 ppm as the ferric potassium ethylene diamine tetra-acetate complex (13). Salts equivalent to the above concentrations were added on June 3, and again on July 14, 1954. Chlorine as KCl, and bromine as KBr were added one time only in the concentrations outlined in table IV. Potassium sulfate (0.5 M) was used to equalize the K concentrations for the various Cl levels. The culture solutions for both experiments were prepared from salts of the analytical grade that tested low in halide. No special purification relative to Cl or Br was found necessary to produce halide deficiency symptoms on sugar beet plants. Except for the salt additions as noted, the solutions were not changed during the course of the experiment. The plants were grown from June 8 to August 2, 1954.

HARVEST: The tops of the plants were separated from the roots at the first leaf scar. The fibrous roots were removed from the storage root, centrifuged for 5 minutes at $39 \times g$, weighed, and dried at 80° C in a forced draft oven. The storage roots were weighed and pulped, and 3 samples weighing 26.0 gm each were frozen with dry ice immediately and analyzed for sucrose at a later date. The tops of the plants were separated into recently matured leaves, i.e., those normally showing the first symptoms of K deficiency, and immature leaves, i.e., those which normally show the first Cl deficiency symptoms. The petioles and blades of these leaves were weighed and dried separately.

In the Cl series experiment the leaf separations were extended to include the living leaves older than the ones matured recently. Only the upper half of each blade, exclusive of the mid-rib, was saved as the "blade" material for chemical analysis. The petiole material did not include the mid-rib of the leaf blade. All leaf material after drying at 70 to 80° C was ground to pass the 40-mesh sieve of a Wiley mill.

ANALYTICAL PROCEDURE, *Chlorine:* The very small amounts of Cl in the plant material of Cl deficient plants made it necessary to adopt the micro-diffusion method for Cl as described by Conway (3). This method was modified for plant material and is based on that used by Broyer et al (2). Ground plant material (0.5 gm for Cl deficient and 0.1 gm for Cl treated plants) was mixed thoroughly with a low halide CaO which was prepared as recommended by Piper (25). The CaO-sample mixture was moistened with water, allowed to stand for 30 minutes, evaporated to dryness and ashed for 15 hours at 500° C in an electric muffle. The ash was dissolved by adding 5.0 ml redistilled water and 5.0 ml of 4.0 *N* HNO_3 and then digesting on the steambath for 5 minutes. The extract was made up to a 25.0 ml volume. An appropriate aliquot of this extract was placed in the outer compartment of a Conway dish and the total sample volume was made up to 2.0 ml with redistilled water. Three ml of freshly prepared 6.7 % KI was added to the center compartment. An oxidant mixture of 30 % H_2SO_4 and 3.0 % $KMnO_4$ (prepared by mixing 15.0 ml of precooled 60 % H_2SO_4 and 15.0 ml of 6 % $KMnO_4$ in an ice bath) was added to the outer

TABLE I

CHEMICAL COMPOSITION OF NUTRIENT SOLUTIONS IN MILLIEQUIVALENTS PER LITER FOR 2^5 FACTORIAL EXPERIMENT *

TREATMENTS	HIGH POTASSIUM SOLUTIONS						LOW POTASSIUM SOLUTIONS					
	K^+	Ca^{++}	Na^+	$SO_4^=$	Cl^-	$SiO_3^=$	K^+	Ca^{++}	Na^+	$SO_4^=$	Cl^-	$SiO_3^=$
0	4.0	5.0	0	2.25	0	0	0.5	8.5	0	2.25	0	0
Cl	4.0	5.5	0	2.25	0.5	0	0.5	8.5	0	2.25	0.5	0
Na	4.0	5.0	0.5	2.75	0	0	0.5	8.5	0.5	2.75	0	0
NaCl	4.0	5.0	0.5	2.25	0.5	0	0.5	8.5	0.5	2.25	0.5	0
Si	4.0	5.0	0	2.25	0	0.5	0.5	8.5	0	2.25	0	0.5
SiCl	4.0	5.5	0	2.25	0.5	0.5	0.5	9.0	0	2.25	0.5	0.5
NaSi	4.0	5.0	0.5	2.25	0	0.5	0.5	8.5	0.5	2.25	0	0.5
NaSiCl	4.0	5.0	0.5	2.25	0.5	0.5	0.5	8.5	0.5	2.25	0.5	0.5

* Both the high and low K solutions also received 7.5 meq NO_3^-, 1.0 meq $H_2PO_4^-$, 2.0 meq Mg^{++}, 0.25 mg Mn^{++}, 0.25 mg B, 0.025 mg Zn^{++}, 0.01 mg Cu^{++}, 0.005 mg Mo and 4.9 mg Fe^{+++} per liter. The iron was added as the ferric potassium ethylene diamine tetra-acetate complex, prepared as recommended by Jacobson (13). Silicon was added either as H_2SiO_3 (suspension) or as Na_2SiO_3 (solution).

Salts equivalent to the foregoing concentrations were added to the 20-l tanks on October 9, 1953 and again on November 24, 1953. Through these additions the low-K and high-K plants received a total of 20 and 160 meq K per 20-l tank, respectively. An extra 0.5 mg Mn^{++}/l was added as $MnSO_4$ to all solutions on December 11, 1953.

compartment. A pre-greased glass lid was placed on the Conway dish immediately after the addition of the oxidant. The samples were covered with a white towel and left on the laboratory bench for 2 hours. At the end of this time the glass lids were removed and the KI solution in the center compartment was transferred by suction to a 5.0-ml volumetric flask. During the transfers, the center compartment was thoroughly rinsed with a 0.4 % starch solution. The flasks were made up to a 5.0-ml volume with a 0.4 % starch solution (3). The absorbance of the starch iodide color complex was determined by a Beckman Model B spectrophotometer with a λ of 540 mμ.

Bromine: The Br analyses were done in 2 parts, 1) an analysis of total halide and 2) an analysis of Br. The difference was assumed to be the Cl content. The analysis of the total halide was similar to the method for Cl analysis with the following exceptions: 1) three ml of a solution of 0.6 % starch and 3.33 % KI was aded to the center compartment, 2) the Conway dishes were shaken gently at 60 cycles per minute for 1.5 hours during the oxidation period, 3) during the transfers the center compartment was rinsed with redistilled water. The analytical procedure for bromine was essentially the same as for the total halide with the following change: 1.0 ml of 40 % K_2CrO_4 was added to the outer compartment followed immediately by 1.0 ml of 80 % H_2SO_4.

Sucrose: The sucrose concentration of the beet roots was estimated in a saccharimeter after preparing a hot water extract of the frozen beet pulp samples as described by Browne and Zerban (1).

Other Elements: Potassium and Na were determined with a Perkin-Elmer flame photometer; NO_3-N by the phenol-disulphonic acid method (15); PO_4-P soluble in 2 % acetic acid by the phospho-molybdate method (33); and SO_4-S by the H_2S-methylene blue procedure (14).

RESULTS

2^5 FACTORIAL: The sugar beet plants for the 2^5 factorial experiment were transplanted at the 2-leaf stage on Oct. 12, 1953 and harvested Jan. 14, 1954. The plants grew exceedingly well at the high K level except for a minor chlorosis of the blades of the immature and recently matured leaves of some of the plants. The symptoms were first observed on December 10, 1953 and were particularly noticeable when the leaves were viewed through transmitted light. At that time the symptoms were thought to be an incipient Mn deficiency which had appeared even though Mn had been added to all nutrient solutions. On December 11 an attempt was made to correct the supposed deficiency by adding 0.5 ppm Mn as $MnSO_4$, to all cultures. However, one week later the symptoms still persisted. A review of all plants in the experiment was made on December 23. This inspection revealed that of the 32 cultures high in K, regardless of variety or the presence or absence of Na or Si, only the plants in the 16 cultures without Cl added showed the symptoms (fig 1). In the 32 cultures low

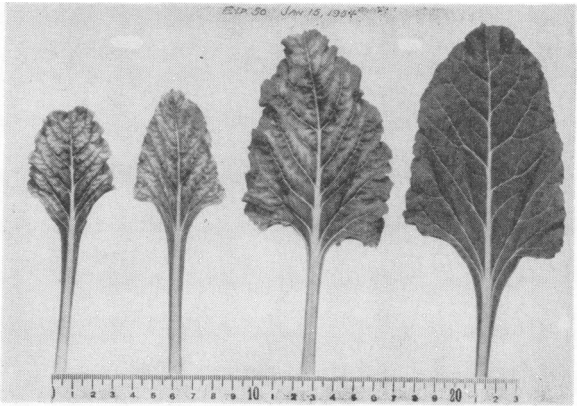


FIG. 1. Blades of sugar beet leaves from Cl deficient plants (*3 leaves on the left*) and from a non-deficient plant (*leaf on the right*).

in K all plants showed the usual K deficiency symptoms on the recently matured leaves, but in addition the center leaves of the plants in 15 out of the 16 cultures without the Cl added showed the same symptoms as the high K plants that were lacking Cl. Thus, the K deficient plants without Cl added showed simultaneously the usual K deficiency symptoms on the recently matured leaves and the Cl deficiency symptoms on the immature center leaves of the sugar beet plant. The immature leaves of the plants low in K but with the Cl added were normal and without symptoms.

The symptoms of Cl deficiency appear first as a chlorosis on the leaf blades of the younger leaves of the plant. The main veins and even the minor veins of these leaves remain green, while the interveinal areas are a light green to yellow in color. Through transmitted light, the leaf blade has a netted mosaic pattern with the branches of the veins forming the netting. Early phases of this pattern are reminiscent of Mn deficiency on sugar beet leaves. As the symptoms develop, the interveinal areas appear as smooth flat depressions, light green to yellow in color, which is in striking contrast to the green veins having a "raised" appearance. The advanced stages of the deficiency for the sugar beet plant are unique for Cl and are clearly distinguishable from the mottling as expressed by Mn, Fe, or Mo deficiency. Another special feature of Cl deficiency for plants grown in culture solution is the very stubby growth of the roots. The many stubs arising from secondary roots form a distinctive and abnormal root structure (fig 2).

In addition to the Cl deficiency symptoms noted on the leaves and roots of the sugar beet plant, the growth of the plant was retarded. These effects of Cl and those of Na, Si, and K are presented in table II as average values for the two sugar beet varieties studied. From the average values and from the F-values of the statistical analyses (30) it is evident that the addition of Cl as the Ca or Na salt (table I) to low Cl culture solutions has resulted in significant increases in the fresh and dry weights of tops and

fibrous roots for plants at both K levels (table II). However, the addition of Cl to high K plants did not increase the size of the storage root. Apparently the time interval prior to harvest was insufficient for the larger tops to effect an increase in root size. In the low K plants adding Cl actually decreased the storage root size instead of increasing it as in the fibrous roots.

The effects of Cl upon the sucrose concentration of the beet roots depend upon the K status of the plants. When plants are high in K, a Cl deficiency causes a decrease in the sucrose concentration of the beet roots, but when the plants are low in K, a Cl deficiency increases the sucrose concentration of the beet roots. This phenomenon can account for the conflicting reports of the effects of K deficiency on sucrose concentration. Thus, when large amounts of Cl are present in the culture solution as an impurity, a K deficiency decreases the sucrose concentration of the beet root but when Cl is low or deficient, a K deficiency increases the sucrose concentration of the beet root.

The Cl concentrations of the petioles of immature and recently matured leaves of Cl deficient plants (table III) are the same as those at the lowest Cl level in the Cl series experiment to be discussed later (see table IV). These values (table III) are approximately the same for both the high and low K plants. Neither the addition of Na, which increased the growth of the low K plants, nor that of Si influenced the petiole Cl concentrations of the plants deficient in Cl. The Cl concentrations of the high Cl-low K plants were much higher than for the high Cl-high K plants. This decrease in Cl concentration is a matter of dilution by growth rather than an interference of Cl absorption by plants high in K. Sodium added to the high Cl plants, except for the Si and NaSi comparison increased the Cl concentration of the petioles regardless of the K level; the increases being more pronounced for the mature than for the immature petioles (table III).

An inspection of the top growth made by the plants at the 2 K and Cl levels reveals an interesting interplay of these 2 elements (table II). Growth of the low K-low Cl plants can be increased for example, from 230 to 343 gm/pot by the addition of Cl, or from 230 to 500 gm/pot by the addition of K. In the presence of adequate amounts of Cl, the addition of K to the low K culture increased the growth from 343 to 693 gm/pot. At first glance, the increase in growth from 230 to 343 gm/pot by adding Cl to the low K culture appears to have extended the effectiveness of K in a manner analogous to the recognized sparing action of Na for low K plants (comparison of Cl, 343, with NaCl, 415). However, the effect of Cl added to the low K plants is not the same as that of Na, since

FIG. 2. Roots of Cl deficient (*upper, left*) and of non-deficient (*upper, right*) sugar beet plants. Roots in center photograph are from Cl deficient plants and those in lower photograph from non-deficient plants. Roots have been set into 4-l beakers for photographing only.

TABLE II

EFFECTS OF CHLORINE, POTASSIUM, SODIUM AND SILICON UPON SUGAR BEET PLANTS

TREATMENT *	TOPS				ROOTS				SUCROSE %		BLADE K %	
	FRESH GM		DRY GM		STORAGE, FRESH GM		FIBROUS, DRY GM					
	HIGH K	LOW K	HIGH K	LOW K	HIGH K	LOW K	HIGH K	LOW K	HIGH K	LOW K	HIGH K	LOW K
					Treatment × K means (4 values)							
0	500	230	52.7	32.5	145	68	2.05	2.10	8.4	10.4	6.42	0.58
Cl	693	343	65.2	38.2	166	43	2.72	4.30	9.3	8.9	5.18	0.57
Na	527	277	58.3	39.9	155	89	3.25	2.55	8.0	9.7	5.37	0.43
NaCl	744	415	72.9	43.9	195	59	4.23	4.76	9.3	9.2	3.91	0.57
Si	529	220	59.1	29.7	174	65	2.88	2.02	9.1	9.8	5.57	0.63
SiCl	735	329	72.1	37.1	191	53	3.71	4.87	10.4	8.2	4.22	0.64
NaSi	537	314	61.0	44.6	216	103	2.49	3.26	8.9	9.1	4.65	0.50
NaSiCl	833	444	84.7	49.5	225	69	6.09	6.27	10.4	9.2	3.55	0.57
LSD (Student) 5 % level	116	74	10.6	7.9	n.s.	21	1.33	1.22	n.s.	n.s.	1.13	n.s.
LSD (Student) 1 % level	160	102	14.6	10.9	n.s.	29	1.83	1.68	n.s.	n.s.	1.55	n.s.
					Variety × K means (16 values)							
U.S. 22/3	608	298	60.5	36.1	175	69	2.94	3.17	8.9	9.4	5.02	0.55
GW 304	667	345	71.0	42.8	191	69	3.91	4.36	9.5	9.2	4.69	0.57
					F-values **							
Cl	69.3	48.9	40.8	8.8	2.0	25.8	23.5	79.5	8.4	10.6	23.4	2.1
Si	2.4	0.4	7.8	0.7	5.7	2.6	5.4	5.6	4.8	2.8	7.4	1.6
Na	2.8	21.9	7.8	29.4	3.7	20.7	14.1	9.6	0.1	0.0	13.6	5.3
Variety	4.7	7.1	17.6	13.2	1.1	0.0	9.6	17.0	1.9	0.3	1.5	0.1

* Cl, Na, Si refer to the high level treatment.

** Required F-values for the one degree of freedom for each treatment, Cl, Si, Na, and Variety, and the 16 degrees of freedom for error are 4.49 and 8.53 for the 5 % and 1 % levels, respectively. The treatment interactions were not significant statistically, except for a few instances at the 5 % level only.

the distinctive Cl deficiency symptoms in the center leaves disappear upon the addition of Cl. A probable explanation for the response by adding either Cl or K to low Cl-low K plants can be found in the different abilities of these 2 ions to be reutilized within the plant. Potassium is readily mobilized from older leaves and moved to newly formed ones, whereas with Cl this does not appear to be the case. Thus, for plants deficient in both K and Cl, added Cl removes the Cl stress of the newly formed center leaves, and the Cl deficiency symptoms disappear. With the Cl stress removed, the younger center leaves utilize the K from the older leaves and more plant growth takes place. When K_2SO_4 was added to low Cl-low K plants Cl (being an impurity in this salt) was added also. Since the quantitative requirement for Cl is considerably lower than for K, both deficiencies were corrected together for a time and extra growth resulted until Cl again became deficient. The Cl added as an impurity from the K_2SO_4 in this experiment was, however, insufficient for continuous growth for high K plants.

The significant effects of Si on the growth of the sugar beet plant are not easy to assess from the results of the present experiment. None of the effects attained the 1 % level of significance but at the 5 % level the effects of Si occurred mainly with the plants high in K. Thus, on the average, the presence of Si, either as the Na salt or as silicic acid increased the dry weight of the tops, the fresh weight of the fibrous roots, and the sucrose concentrations of the beet roots. In the low K plants only the weights of the fibrous roots were significantly increased by the addition of Si to the culture solution.

In accord with the findings of other investigators, the addition of Na to low K plants increased significantly the weight of the tops and the weight of the storage and fibrous roots (table II). The sucrose concentrations of the storage roots were not changed appreciably by the Na treatments for either the low or high K plants. For the plants high in K, the addition of Na increased the dry weight of the tops and the dry weight of the fibrous roots but had no significant effect upon the weights of the fresh tops or upon the beet roots.

The GW 304 variety made significantly better top growth and had more fibrous roots than the U.S. 22/3, when grown at either K level (table II). Neither variety, however, differed significantly in beet root weight or in sucrose concentration. Nor were there

TABLE III

PETIOLE CHLORIDE (DRY BASIS) AS AFFECTED BY AGE OF LEAF AND POTASSIUM, SODIUM, SILICON AND CHLORINE NUTRITION

TREATMENT *	YOUNG PETIOLES		MATURE PETIOLES	
	HIGH Cl PLANTS μEQ/GM	LOW Cl PLANTS μEQ/GM	HIGH Cl PLANTS μEQ/GM	LOW Cl PLANTS μEQ/GM
Treatment × age × Cl means (4 values)				
0	410	7.3	444	9.9
K	133	6.7	305	8.9
Si	483	7.9	528	11.7
SiK	141	5.8	300	7.2
Na	457	6.1	558	9.5
NaK	275	6.2	513	6.9
NaSi	477	5.7	490	9.8
NaKSi	198	6.7	378	8.9
Cl × age means	322	6.5	439	9.1
LSD (Student) 1 % level	176	n.s.	171	n.s.
Variety × age × Cl means				
U.S. 22/3	340	6.6	462	9.1
GW 304	304	6.5	417	9.1
F-values **				
K	80.5	0.5	19.8	5.7
Si	0.0	0.0	1.1	0.4
Na	4.0	1.4	9.5	0.5
Variety	1.4	0.0	2.4	0.0

* K, Na, Si refer to the high level treatment.
** See table II for required F-values.

any significant variety-nutrient interactions, indicating that both varieties performed similarly at either Cl, Na, K, or Si level.

GROWTH AS A FUNCTION OF CHLORIDE OR BROMIDE SUPPLY: The results of the factorial experiment showed that Cl ions increased growth when added to modified Hoagland's No. 1 culture solution (table I) prepared from chemically pure salts. The growth increases were accompanied by much higher Cl concentrations within the plant than found in comparable plants without Cl added to the culture solutions. The exact amount required to overcome a Cl deficiency was investigated in the next experiment.

The plants were again grown in culture solutions to which increasing amounts of Cl ion, as KCl, were added to the basic culture solution. As before, a basic culture solution prepared from chemically pure salts and distilled water served as a control. Approximately 0.013 meq Cl as the Cl ion was present as an impurity in a total of 20 l of solution.

In addition to the Cl series of plants another set of cultures was prepared containing KBr at a concentration of 0.001 M (treatment 10, table IV). In treatment 11 the plants received both Cl and Br at 0.001 M concentrations. All plants were grown from June 8 to August 2, 1954. On August 2 the plants were harvested, and the results obtained for each Cl level and Br addition are given in table IV. The Cl concentrations of the petioles and blades of recently matured leaves and their relation to plant growth and sucrose content are given in figures 3, 4, 5, and 6.

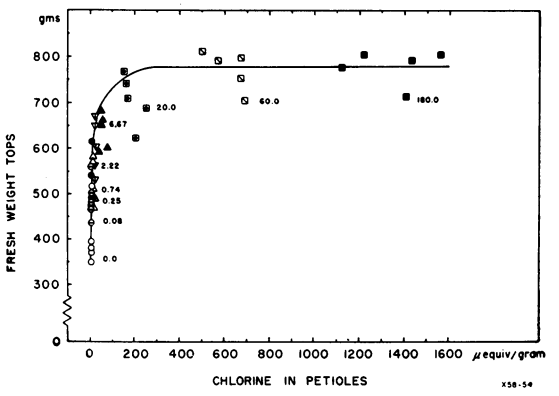
The addition of Cl as Cl^- to the culture solutions again had a large effect upon the growth of the sugar beet plants (table IV). The tops increased from an average value of 402 gm per tank for the solutions containing only 0.013 meq of Cl per 20 l as an impurity (treatment 1) to 705, 771, and 776 gm for solutions that received 20, 60 and 180 meq Cl per 20 l, respectively (treatments 7, 8, and 9). The addition of Br as Br^- at a concentration of 20 meq per 20 l (treatment 10) resulted in a top growth equal to that of the comparable Cl^- treatment (treatment 7). With both Br and Cl present at 0.001 M concentrations, the top weights were again equal to treatments 7 and 10.

The dry weights of the tops are similar in pattern

TABLE IV

EFFECTS OF CHLORINE AND BROMINE ON GROWTH, SUCROSE CONCENTRATION OF STORAGE ROOTS AND ON THE PETIOLE AND BLADE CHLORINE OF SUGAR BEET PLANTS (MEANS OF 5 REPLICATIONS)

TREATMENT			TOPS		OLD LEAVES DRY	STORAGE ROOTS			FIBROUS ROOTS		CHLORINE	
No.	Cl ADDED	Br ADDED	FRESH	DRY		FRESH	SUCROSE		FRESH	DRY	MATURE PETIOLES	MATURE BLADES
	meq	*meq*	*gm*	*gm*	*gm*	*gm*	*gm*	*%*	*gm*	*gm*	*μeq/gm*	*μeq/gm*
1	0.00	0.0	402	48.4	4.62	269	26.4	9.8	48	4.00	4.9	3.3
2	0.08	0.0	489	59.1	4.09	291	30.5	10.4	54	4.58	6.4	5.0
3	0.25	0.0	522	61.6	5.26	359	38.6	10.8	66	5.50	8.1	4.6
4	0.74	0.0	523	61.8	4.32	376	40.9	10.9	55	4.82	12.4	5.4
5	2.22	0.0	603	68.0	4.11	379	42.3	11.2	67	5.71	23.6	8.9
6	6.67	0.0	636	72.3	4.82	446	51.1	11.4	79	6.49	54.0	13.9
7	20.00	0.0	705	73.6	4.62	464	48.1	10.4	82	6.45	185.7	29.7
8	60.00	0.0	771	81.5	5.93	423	44.7	10.5	99	7.76	617.9	95.4
9	180.00	0.0	776	76.1	4.38	517	52.0	10.0	90	6.79	1345.1	269.7
10	0.00	20.0	727	78.3	5.15	415	43.9	10.6	83	6.83	11.0	4.4
11	20.00	20.0	737	76.6	5.69	498	53.8	10.8	94	7.59	179.7	25.7
LSD (Student) 1 % level			97	9.2	n.s.	78	9.2	0.9	24	1.98	....	....



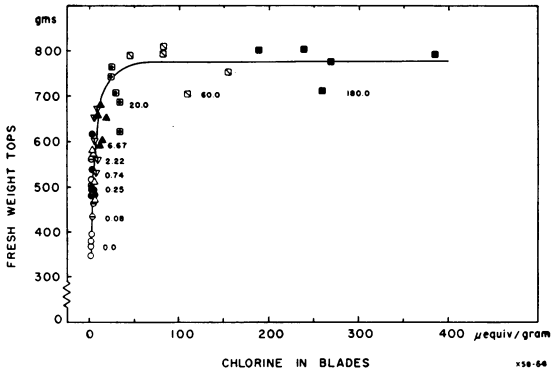


FIG. 3 (*above*). Relation of petiole Cl values of recently matured leaves (dry basis) to the fresh weight of the tops. The numbers in the graph refer to mg atoms of Cl added per 20 l of nutrient solution. The critical petiole Cl concentration, below which top growth decreases, is approximately 200 μeq/gm dry tissue.

FIG. 4 (*below*). Relation of Cl values for the upper upper half of the blades of recently matured leaves to the fresh wt of the tops. The critical blade Cl concentration, below which top growth decreases, is approximately 50 μeq/gm dry material. See also figure 3.

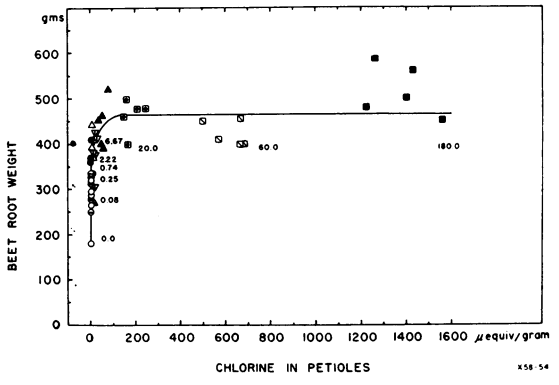
to the fresh weights. The dry weights of the old leaves did not differ significantly from each other.

The beet root weights, in contrast to the results of the factorial experiment, were nearly doubled by the higher Cl additions to the culture solutions. The addition of Br^- at a concentration of 0.001 M to the basic culture solution (treatment 10) resulted in a lower beet root weight than in the comparable Cl concentration (treatment 7) but the decrease was not significant statistically.

The fresh and dry weights of the fibrous roots followed a pattern similar to the storage root weights (table IV). Whereas the appearance of the storage roots was not altered by a Cl deficiency the fibrous roots of deficient plants had many stubby lateral branches in contrast to the filamentous character of roots from plants well supplied with chlorine (fig. 2). The substitution of Br for Cl resulted in fibrous roots that had the same appearance and weight as the Cl treated plants.

Particularly interesting was the observation that when Br^- was added to low Cl solutions there was a complete absence of Cl deficiency symptoms and the growth of tops and roots was excellent. Chloride contamination of the Br salts is hardly the explanation for this good growth, since there was none found in the KBr salts added to the culture solutions. The blade Cl value of 4.4 μeq/gm (treatment 10, table IV) is far below that found for non-deficient plants and the petiole Cl value of 11.0 μeq/gm would indicate a top yield of only 522 gm (treatment 4, table IV) instead of the 727 gm found for the tops of the Br treated plants (treatment 10). Similar comparisons can be made for storage and fibrous root growth. Apparently, these results indicate that Br^- can substitute for Cl^- in the growth of tops and roots.

The Cl values for the petioles and blades of plants in treatment 11 (Cl + Br) agree with those for treatment 7 which received Cl only. The addition of Br to Cl at this concentration did not influence Cl absorption.



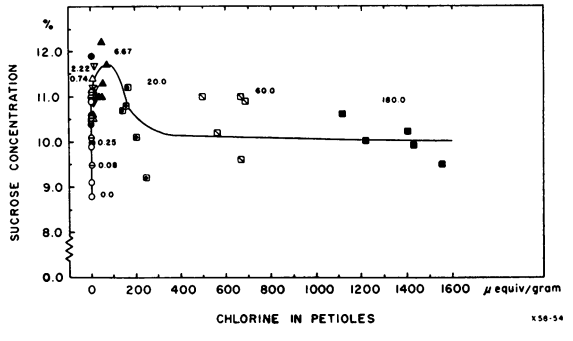


FIG. 5 (*above*). Relation of petiole Cl values of recently matured leaves (dry basis) to the fresh weight of storage roots. The critical petiole Cl concentration, below which storage root growth decreases, is approximately 200 μeq/gm dry material. See also figure 3.

FIG. 6 (*below*). Relation of petiole Cl values of recently matured leaves (dry basis) to the sucrose concentration of storage roots. Sucrose concentrations of the storage root are depressed by an extreme Cl deficiency, increased sharply by a mild deficiency, and lowered by an ample supply of Cl. See also figure 3.

GROWTH AS A FUNCTION OF PETIOLE AND BLADE CHLORIDE CONCENTRATION: The Cl concentrations in the petioles of recently matured leaves are given in table IV as μeq per gram of dry substance. A statistical analysis of the logarithms of the concentrations indicate that a difference of 0.14 expressed as a common log is necessary for significance at the 1 % level. On this basis the values for treatments 1 and 2, and 2 and 3 are alike, but there is a significant difference between 1 and 3. Significant increases in Cl concentrations took place between treatments 4 through 9. For the blades, the Cl values for the first 3 treatments did not differ significantly from each other. Each increment of Cl added thereafter produced a significant increase in Cl concentration in most instances (table IV).

The relation of petiole Cl to top growth is given in figure 3. Top growth begins to be retarded at petiole Cl concentrations of 100 to 200 μeq/gm (critical concentrations) and pronounced deficiencies are indicated by values of less than 10 μeq/gm. Plants with petioles containing 200 μeq/gm and above are adequately supplied with Cl (fig 3 and table IV). Similar conclusions about top growth and Cl concentrations can be drawn from the values for the leaf blades (fig 4 and table IV). The critical range is from 25 to 50 μeq/gm of dry blade tissue; approximately ¼ that of the petioles. For extreme Cl deficiencies the Cl concentrations of the blades are only slightly less than those of the petioles but at the higher Cl levels, the blade values are only 1/5 that of the petiole values. Thus the petioles are far more sensitive to Cl changes within the beet plant than the corresponding blades, and therefore petiole analysis appears preferable to blade analysis as a means of measuring the chlorine status of sugar beet plants.

The relation of petiole Cl to storage root growth is given in figure 5. The Cl values relative to storage root growth are quite similar to those for top growth. The critical Cl value is again approximately 200 μeq of Cl per gram of dry tissue, with the plants above this being well supplied with Cl and those with values of less than 10 μeq/gm indicating an extreme Cl deficiency.

PETIOLE AND BLADE BROMIDE CONCENTRATIONS: The petioles of the plants receiving Br only (treatment 10) contained 87.1 μeq of Br per gm of dry plant tissue (average of 5 replications) and the corresponding blades 11.0 μeq/gm. The Br values for the Cl plus Br treatment (treatment 11) are 157.9 μeq/gm for the petioles and 35.1 μeq/gm for the blades. As would be expected, the combined Cl and Br values correspond to those which lie between treatments 7 and 8 for Cl additions alone (table IV). However, still unexplained are the much higher Br concentrations for the petioles of the plants treated with Cl and Br (treatment 11) than for those treated with Br only (treatment 10). One explanation is that this is just a chance observation that is associated with the plant and another explanation is that it is associated with analytical variation but this is very unlikely because the values for duplicate samples agreed very closely with each other. While the yields for the Br treated plants are somewhat higher than what would be expected for a petiole halide concentration of 98.1 μeq/gm (between treatments 6 and 7, table IV) this may be due to chance and not to a real effect of Br.

TABLE V

NUTRIENT GRADIENTS WITHIN SUGAR BEET LEAVES EXPRESSED AS MICROGRAM ATOMS PER GRAM DRY TISSUE

PLANT PART	Cl	SO_4-S	NO_3-N	PO_4-P*	K	Na
Upper blade ...	78	151	46	77	396	1500
Lower blade ...	366	128	106	82	407	1710
Mid-rib of blade	694	63	436	54	417	1820
Upper petiole ..	938	21	715	72	524	1780
Middle petiole	1079	20	680	70	570	1750
Lower petiole ..	1262	24	609	72	650	1830
Entire blade ..	260	126	169	68	417	1610

* Phosphate-phosphorus soluble in 2 % acetic acid.

SUCROSE FORMATION RELATIVE TO CHLORIDE OR BROMIDE SUPPLY: As may be seen in table IV and figure 6, Cl deficient plants are significantly lower in sucrose concentration than comparable plants with an adequate supply of this element. Apparently, Cl is involved in the formation of sugar rather than in sucrose utilization. If Cl had been related to sugar utilization, then a deficiency of Cl would have caused an increase in sucrose concentration of the beet root, analogous to that of a N deficiency (34). Increasing the Cl supply still more, however, leads to a decrease in sucrose concentration of the storage root (treatments 7, 8, and 9). The cause of this decrease is not known.

Replacing Cl by Br (treatment 10, table IV) or by adding Br and Cl salts together at equal concentrations (treatment 11), caused no significant change in the sucrose concentration of the beet roots (treatments 7, 10 and 11). This indicates that Br may substitute for Cl not only in top and root growth but also in maintaining the sucrose concentration of the beet roots.

DISTRIBUTION OF CHLORIDE WITHIN THE PLANT: Within the tops of plants adequately supplied with Cl, the Cl concentrations of the blades differed little with respect to age of the leaf, being 51, 45 and 65 μeq/gm for young, mature and old leaves, respectively. In the petioles of the same leaves the Cl concentrations were very much higher than in the blades, being 279, 470 and 701 μeq/gm for the young, mature and old leaves, respectively. This increase in Cl concentration with the age of the petiole has been found to be typical of sugar beet plants studied thus far.

The chloride gradient within the leaves of field grown plants is indicated in table V for leaves separated into upper blades, lower blades, mid-ribs, and upper, middle, and lower sections of petioles. Within the petiole the Cl concentration decreases slowly from the base of the petiole to the mid-rib. The mid-rib itself, while appreciably lower in Cl than the petiole,

is much higher in Cl than the blade tissues. The upper blade is distinctly lower in Cl than the lower part of the blade. The reason for the sharp decrease in Cl concentration from the petiole to the blade tissues is not known, although the selective absorption by the petioles or the exclusion of Cl by the blade tissues could give rise to such concentration differences. A similar gradient for NO_3-N exists (table V), but here the decreases are quite likely associated with nitrate reduction and not with absorption barriers as seems to be indicated for the movement of Cl from conducting to blade tissues.

The marked decrease in Cl concentration from petioles to blades is apparently unique among the mineral constituents of sugar beet leaves (table V). Phosphate-phosphorus tends to be higher in the blades than in the petioles, especially when there is a P deficiency and when the plants are deficient in N (34). Similarly, when plants are deficient in K and contain ample quantities of Na, K concentrations of the blades are higher than in the petioles, but when the plants are deficient in other nutrients, the petioles frequently increase in K concentration far more rapidly than the blades. Sodium concentrations in these plants differ little from petioles to blades. In contrast to the Cl concentrations, SO_4-S concentrations are far higher in the blades than in the petioles.

CHLORIDE RECOVERY: The addition and recovery of Cl ions from sugar beet plants was studied in an experiment that involved only 2 treatments. In one treatment sugar beet plants were grown in modified Hoagland's culture solution which contained all elements known to be necessary to plant growth, including Na and Si but not Cl. The plants in the other treatment received the same nutrients, except for 20 meq of $SO_4^=$ from Na_2SO_4, which was replaced by 19.7 meq of Cl^- from NaCl. Sugar beet plants of the U.S. 22/3 variety at the early 2-leaf stage were set into the culture solutions on February 5, 1954. On April 22, 1954 the plants were separated into tops and roots. These parts were dried at 80° C and analyzed for Cl in triplicate by the modified Conway procedure.

The Cl recovered from the plants in each treatment is given in table VI. The tops and roots of the plants low in Cl contained 0.380 meq of Cl. The original culture solution contained 0.040 meq and the seedlings at the time of transplanting 0.009 meq or a total of only 0.049 meq of Cl. At the 1 % level, the lower confidence limit for Cl recovery in the plant is 0.250 meq of Cl. This value exceeds by far the Cl originally present (0.049 meq) as a contaminant. This implies that the plants accumulated Cl during the growth period from the distilled water added from time to time to the culture solutions or from other sources such as the air surrounding the plants. Since the distilled water supplied at most only 0.005 meq of Cl, the rest must have originated elsewhere.

The Cl recovered from the plants receiving 19.7 meq of Cl averages 19.8 meq (table VI). After subtracting 0.4 meq of Cl recovered from the minus Cl plants, a net recovery of 19.4 meq of Cl is obtained, which compares favorably to the amount added initially, 19.7 meq. The confidence limits for the net recovery of Cl from 6 sets of plants are 19.3 and 20.3 meq at the 5 % level and 19.0 and 20.7 meq at the 1 % level. This implies that there is a probability of 95 and 99 %, respectively, that these limits contain the true mean of Cl recovery for these plants. Since these limits cover the amount of Cl added initially, one may conclude that there has not been an appreciable absorption of extraneous Cl or a loss of Cl from the plant system, unless the losses and gains cancelled each other or occurred within the confidence limits of the present experiment.

CHLORINE DEFICIENCY SYMPTOMS ON PLANTS GROWN IN HOAGLAND'S NUTRIENT SOLUTION: The ease with which Cl deficiency symptoms can be induced in sugar beet plants, using technical grade chemicals, regular distilled water, and vermiculite as a substrate came as a surprise when demonstrating the growing of sugar beet plants by the "open culture" technique. In the demonstration, sugar beet seeds were planted in vermiculite at a depth of ¾ inches in November 1953 and thereafter watered daily with approximately one liter of half strength Hoagland's nutrient solution No. 1 (11). The excess solution drained off freely and was not recovered for later use. When the plants were 6 to 8 weeks old, symptoms similar to those illustrated in figure 1 developed on the blades of the young leaves. On February 24, 1954 the pots were divided into 2 lots. One lot was watered thereafter with half strength Hoagland's solution to which $CaCl_2$ at a concentration of only 0.1 meq of Cl per liter had been added, the other lot watered as before without the $CaCl_2$ addition. The deficiency symptoms were completely removed within

TABLE VI

CHLORINE RECOVERY FROM SUGAR BEET PLANTS

POT	TOPS DRY WT GM	ROOTS DRY WT GM	TOPS Cl μEQ/GM	ROOTS Cl μEQ/GM	TOPS Cl MEQ	ROOTS Cl MEQ	TOTAL Cl MEQ
			High chlorine plants (19.7 meq Cl^- added)				
69	107.9	101.6	179.2	7.88	19.34	0.80	20.14
72	118.5	67.8	160.3	11.66	19.00	0.79	19.79
85	109.0	115.0	169.7	10.00	18.50	1.15	19.65
102	105.9	69.9	175.0	9.55	18.53	0.67	19.20
119	112.5	138.7	163.7	12.99	18.42	1.80	20.22
Mean	110.8	98.6	169.6	10.42	18.76	1.04	19.80 *
			Low chlorine plants (0.049 meq Cl^- impurity)				
80	62.6	62.5	3.95	1.90	0.25	0.12	0.37
88	91.1	75.0	3.58	1.04	0.33	0.08	0.41
93	62.3	53.4	4.23	0.94	0.26	0.05	0.31
106	89.1	93.8	3.89	1.22	0.35	0.11	0.46
113	65.4	70.3	3.85	1.19	0.25	0.08	0.33
Mean	74.1	71.0	3.90	1.26	0.29	0.09	0.38 **

* Confidence limits at the 5 % level, 19.29 to 20.31.
** Confidence limits at the 5 % level, 0.30 to 0.46

25 days after the addition of Cl^-. Those plants without the Cl added continued to retain and develop leaves with deficiency symptoms.

In the culture of sugar beets, it is recommended that Cl at a concentration of 5.0 meq per liter be added to Hoagland's solution either as $CaCl_2$ or as NaCl. The addition of NaCl is preferable to $CaCl_2$ whenever "normal" nutrition in relation to potassium and sodium is desired (unpublished results of this laboratory).

Discussion

Failure to observe chlorine deficiency symptoms on sugar beet plants earlier was undoubtedly due to the presence of Cl as a contaminant in the salts used to prepare the nutrient solutions and perhaps to the use of unfiltered air in contrast to greenhouse air now filtered through activated carbon. Still another factor is the similarity of the incipent Cl deficiency symptoms to those of manganese deficiency.

In the factorial experiment, involving Cl, Na, Si, K and variety of sugar beet, the first symptoms, which later proved to be associated with Cl, were visible only through transmitted light. These symptoms appeared on young and recently matured leaves and were similar to those of Mn deficiency. Even after adding 10 mg of Mn as $MnSO_4$ to each tank of culture solution, the deficiency symptoms persisted. A more careful inspection of the plants revealed that the symptoms appeared only on those plants to which no Cl had been added to the culture solution. As these plants became older symptoms could be seen readily on the high K-low Cl plants, and somewhat less readily on the center leaves of the low K-low Cl plants. If the K salts had contained more Cl as an impurity, the Cl deficiency symptoms would not have been observed in the low K plants nor even in the high K plants, since by adding K as K_2SO_4, Cl as a contaminant would have been added at the same time. One may assume that as the manufacturers of reagent and technical grade salts have improved their processes, salts lower in Cl have been produced, and now Cl must be added to nutrient solutions to prevent Cl deficiency of sugar beet plants.

Summary

Sugar beet plants were grown in nutrient solution prepared from low halide salts of the reagent grade. The first deficiency symptoms developed on the blades of the center leaves of the plant and in the early stages resembled those of Mn deficiency. Through transmitted light the symptoms appeared as a netted mosaic pattern, with the green veins forming the netting. As the symptoms developed, the interveinal areas appeared as smooth flat depressions, light green to yellow in color, and presented a striking contrast to the green veins which had a "raised" appearance. A feature of the Cl deficiency was the stubby growth of the secondary roots. Both the leaf and root symptoms were absent in culture solutions having the additions of Cl in the amounts of 6.7 to 20 meq per three plants and Br at the one amount used, 20 meq per three plants. The addition of Mn, Na, Si, as well as those salts contained in Hoagland's solution No. 1 failed to correct the symptoms.

Petiole Cl values of 4.9 to 7.9 μeq and blade Cl values of 3.3 to 5.4 μeq per gram of dry tissue were indicative of extreme Cl deficiency. Petiole Cl values of 200 μeq or above and blade (without mid-ribs) Cl values of 50 μeq or above per gram of dry tissue were indicative of Cl adequacy.

Chlorine was necessary for top and root growth and was associated with sugar formation rather than with sugar utilization. The storage roots of plants deficient simultaneously in Cl and K were higher in sucrose concentration than non-deficient plants, whereas a deficiency of either nutrient alone resulted in a decrease in sucrose concentration. Still more Cl, however, depressed sucrose concentrations of the storage root but did not decrease root size.

Petiole Cl concentrations, but not those of the blades, increased with the age of the leaf. For plants well supplied with Cl, the Cl concentrations within the upper blade tissues of the leaf were less than one-tenth those of the corresponding petioles. Comparable decreases for NO_3-N were observed in the leaf blades, but this decrease was due to nitrate reduction, whereas the Cl decreases were thought to be associated with absorption barriers or to factors not now known. Sulfate, in contrast to Cl and NO_3, accumulated in blade tissues at far higher concentrations than in the corresponding petioles.

The Cl recovered from untreated plants was in excess of the Cl present as an impurity in the nutrient solutions and that contained in the transplanted seedlings, whereas complete recovery of chloride was indicated for plants treated with 20 meq of Cl after correcting for the Cl found in the untreated plants.

Chlorine deficiency symptoms were induced in sugar beet plants that were grown in the greenhouse by the "open culture" technique, using nutrient solutions prepared from chemically pure salts without special purification, regular distilled water, and vermiculite as a substrate.

The addition of Na to low K plants increased growth significantly but had no effect upon high K plants. The effects of silicon were not conclusive.

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THE LARGE SCALE LABORATORY CULTURE OF CHLORELLA UNDER CONDITIONS OF MICRONUTRIENT ELEMENT DEFICIENCY[1]

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Elucidating the metabolic roles of the micronutrient elements through analysis of both normal and deficient tissues for products of metabolism and for enzymes may provide clues as to the function of the various elements.

The unicellular alga, Chlorella, was chosen as a test organism for this purpose because it can be grown readily under controlled concentrations of micronutrients and can be sampled quite accurately. A survey of the literature revealed no extant Chlorella culture apparatus which was easily adaptable for the

[1] Received November 15, 1955.