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ANATOMICAL AND PHYSIOLOGICAL RESPONSES OF THE TOMATO TO VARYING CONCENTRATIONS OF SODIUM CHLORIDE SODIUM SULPHATE, AND NUTRIENT SOLUTIONS

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(WITH TEN FIGURES)

Introduction

Plants grown in soil solutions containing high concentrations of soluble salts commonly exhibit markedly unfavorable growth responses. These may be caused by (u) the toxic effect of high concentration of a single ion, (b) the interaction of two or more ions present in high concentrations, or (c) the combined effect of high total salt concentration.

Experiments were set up to determine (a) the character of the anatomical and physiological responses of tomato plants when grown in a series of nutrient solutions containing progressively higher concentrations of the major constituent salts, and (b) the responses these plants exhibit when grown in sodium chloride and sodium sulphate solutions having osmotic concentrations equal to those of the solutions in the nutrient series.

Material and methods

The tomato plant was selected because it can be easily grown under greenhouse conditions and has been used extensively in studies of nutrition. The plants were grown in sand cultures in pots devised by EATON (2). These are provided with a reservoir holding approximately 13 liters of solution, which can 'be circulated at regular intervals by means of compressed air passing through an ejector. A gage on the rkservoir indicates the level of the solution, and water was added daily to compensate for losses resulting from transpiration and evaporation. A time clock controls flushing of the cultures, and was set to circulate the liquid at 2-hour intervals from 6:00 A.M. to 6:00 P.M., with one irrigation at midnight. A flow for 3-5 minutes more than displaces the solution held in the sand.

NUTRIENT AND SALT SOLUTIONS

EXPERIMENT I.--A four-salt nutrient solution was used containing $Ca(NO_3)_2$, KNO_3, $MgSO_4$, and KH_2PO_4, and the total salt concentration was adjusted to 0.5 atmosphere osmotic concentration (table I). This solution was used as the base

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nutrient in the controls and as the initial solution for all cultures until the seeds had germinated. It also constituted the nutrient portion of the sodium chloride and sodium sulphate solutions.

Three series of solutions were used:

1. Base nutrient series: Four solutions in which the nutrient salts were increased at each level to produce osmotic concentrations of 0.5, 1.5, 3, and 4.5 atmospheres.
2. Sodium chloride series: Three solutions consisting of the base nutrient at 0.5 atmosphere plus NaCl sufficient to result in osmotic concentrations of 1.5, 3, and 4.5 atmospheres.
3. Sodium sulphate series: Three solutions consisting of the base nutrient at 0.5 atmosphere plus Na_2SO_4 sufficient to result in osmotic concentrations of 1.5, 3, and 4.5 atmospheres.

TABLE 1

COMPOSITION OF SOLUTIONS USED IN EXPERIMENT I

Concentration of solutions (atmospheres)	Base nutrient series consisting of following salts (millimoles per liter)				Concentration of solutions (atmospheres)	Millimoles added to base nutrient solution at 0.5 atmosphere concentration	
	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4		Sodium chloride series NaCl	Sodium sulphate series Na_2SO_4
0.5	2.6	5.2	2.6	0.65	1.5	20.0	15.0
1.5	7.2	74.4	7.2	1.8	3.0	52.0	40.0
3.0	15.8	31.6	15.8	3.95	4.5	89.0	71.0
4.5	24.8	49.6	24.8	6.2			

The proportions of the constituent salts used in the various solutions are expressed in millimoles per liter. In determining the amounts of salts needed to produce 0.5 atmosphere osmotic concentration, c.p. salts and distilled water were used. When commercial salts and Riverside tap water were used, the proportions produce an osmotic concentration of approximately 0.6 atmosphere. Minor elements were added in the following amounts: boron 1.0 p.p.m., zinc 0.05 p.p.m., manganese 0.5 p.p.m., molybdenum 0.05 p.p.m., and copper 0.02 p.p.m. Iron as citrate was supplied as needed in 1 p.p.m. additions.

EXPERIMENT II.—The solutions used were essentially like those in experiment I, but the quantities of salts needed to produce 0.5 atmosphere osmotic concentration were determined on the basis of commercial salts and tap water. The amount of KH_2PO_4 was kept at a constant level (1.8 mm./l.) in all solutions, to avoid the heavy precipitation that occurred in the higher base nutrient cultures in experiment I. In order to explore further the effect of high salt concentrations, an additional culture with salts adjusted to 6 atmospheres osmotic concentration was added to each series. The base nutrient series was set up at five levels: 0.5, 1.5, 3, 4.5, and 6 atmospheres osmotic concentration; and the sodium chloride and sodium sulphate series at four levels: 1.5, 3, 4.5, and 6 atmospheres. The composition of the solutions is indicated in table 2. Minor elements and iron were added as in experiment I, and additional increments of manganese (0.5 and 1 p.p.m.) were supplied during the latter part of the run, when symptoms of manganese deficiency appeared in the leaves of the larger plants.

TABLE 2

COMPOSITION OF SOLUTIONS USED IN EXPERIMENT II

Concentration of solutions (atmospheres)	Base nutrient series consisting of following salts (millimoles per liter)				Concentration of solutions (atmospheres)	Millimoles added to base nutrient solution at 0.5 atmosphere concentration	
	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4		Sodium chloride series NaCl	Sodium sulphate series Na_2SO_4
0.5..	1.3	2.6	1.3	1.8	1.5.........	20.0	15.0
1.5.........	7.3	14.6	7.3	1.8	3.0.........	54.0	40.0
3.0..........	16.1	32.2	16.1	1.8	4.5.........	90.0	71.0
4	26.0	52.0	26.0	1.8	6.0.........	120.0	100.0
6.0..........	35.1	70.2	35.1	1.8			

REPLACEMENT AND MAINTENANCE OF SOLUTIONS

To avoid seedling injury, at the initiation of each experiment all pots were supplied with the base nutrient at the 0.5 atmosphere level, and the higher osmotic concentrations were obtained by a series of increases extending over a 10-day period. Complete changes of solutions were made as follows : experiment I-October 5 and 23, and November 7; experiment II-November 21, December 7 and 26, and January 7. At intervals between solution changes the osmotic concentration of each solution was determined by the freezing-point depression method.

The H-ion concentration was determined with a Beckman pH meter on alternate days, or daily when the plants were larger. Solutions were adjusted to pH 7.0 by additions of HNO, or NaOH when there was a variation of more than 0.5 pH. In experiment II, KOH was used instead of NaOH. Until the plants were large, it was never necessary to add more than 5 cc. of HNO,, and 10 cc. was the greatest amount supplied at one time.

ENVIRONMENTAL FACTORS (EXPERIMENTS I AND II)

The cultures were grown under greenhouse conditions, and records of temperature and relative humidity were obtained with a hygrothermograph. During the course of experiment I (September 23 to November 11, 1939) the mean maximum temperature was 88.6° and the mean minimum 61.6° F. Up to the time of the first harvest in experiment II (November 15, 1939 to January 2, 1940) the mean maximum and minimum temperatures were only slightly less than those of experiment I, 86.5° and 59.4° F., respectively. The final 4 weeks of the second experiment (January 2 to 30, 1940) were cooler, 78° and 58.1° F. The mean values for relative humidity were: experiment I-maximum 70.9 per cent, minimum 28.7 per cent; experiment II-77.8 per cent and 39.6 per cent up to the first harvest and 81.3 per cent and 56.1 per cent for the final 4 weeks.

The major climatic variation between the two experiments was the amount of solar and sky radiation. At Riverside, California, the mean total daily radiation for the period of the first experiment was 354.8 gm. calories per square centimeter of horizontal surface, while for the equivalent period of the second experiment it was 256 gm. calories. There was a further reduction in radiation during the final 4 weeks of the second experiment when the daily mean was 221 gm. calories.[3] These values were taken outdoors, and somewhat lower ones would obtain in the greenhouse, where the experiments were conducted.

Planting data (experiments I and II)

A commercial strain of the Marglobe variety of the tomato was used, and all trials were run in duplicate.

The seeds of experiment I were planted September 23, 1939, and the cultures given identical amounts of water and nutrient solution until September 30. At this time all except the control pots were increased to 1.5 atmosphere osmotic concentration. On October 3, all but the controls and 1.5 atmosphere cultures were adjusted to 3 atmosphere concentration; and on October 5, the 4.5 atmosphere cultures were brought to full strength. In this manner the three series were established as follows: the base nutrient series at 0.5 ,1.5, 3, and 4.5 atmospheres osmotic concentration and the sodium chloride and sodium sulphate series at 1.5, 3, and 4.5 atmospheres.

The seeds of experiment II were planted November 15, and on November 25 all except the control pots were adjusted to 1.5 atmosphere concentration. Increases in the concentration of the cultures were made as in experiment I. The 3 atmosphere level was reached November 27, the 4.5 on November 29, and the 6 on December 1. The final adjustment established the three series at the following levels: the base nutrient at 0.5, 1.5, 3, 4.5, and 6 atmospheres osmotic concentration and the sodium chloride and sodium sulphate series at 1.5, 3, 4.5, and 6 atmospheres.

3 These figures supplied through the courtesy of Dr. E. R. Parker, at Citrus Experiment Station, Riverside, where a pyroheliometer is maintained.

Experimentation

SEEDLING RESPONSES

Approximately twenty seedlings were started in each culture. These were thinned selectively to provide three uniform plants. Although the seedlings removed were the least uniform in each culture, their average heights and total fresh and dry weights did provide an index of the early responses to the nutrient and salt solutions used in the three series.

TABLE 3

EXPERIMENT II: SEEDLING HARVEST DATA. FRESH AND DRY WEIGHTS AND PERCENTAGE OF DRY MATTER

Series and concentration of solutions (atmospheres)		Harvest December 7, 1939			Harvest December 13, 1939		
		Fresh wt. 12 plants (gm.)	Dry weight (gm.)	Dry matter (per cent)	Fresh wt. 6 plants (gm.)	Dry weight (gm.)	Dry matter (per cent)
Base nutrient	0.5	11.53	0.84	7.3	22.95		7.9
	1.5	13.66	1.11	8.1	27.00	1.99	7.4
	3.0	9.05	0.76	8.5	19.31	1.60	8.3
	4.5	5.25	0.47	9.0	6.61	0.64	9.7
	6.0	5.14	0.51	10.0	6.72	0.72	10.8
NaCl	1.5	9.84	0.70	7.2	17.53	1.33	7.6
	3.0	7.05	0.45	6.4	13.24	0.68	5.2
	4.5	7.85	0.55	7.1	12.24	0.72	5.9
	6.0	6.75	0.41	6.1	8.37	0.63	7.6
Na_2SO_4	1.5	8.43	0.67	8.0	20.28	1.54	7.6
	3.0	7.72	0.60	7.9	12.17	0.77	6.4
	4.5	5.80	0.46	8.0	6.24	0.54	8.8
	6.0	3.59	0.31	8.9	3.15	0.28	9.1

In both experiments there was a progressive growth depression with increasing concentration in the sodium chloride and sodium sulphate series. In the base nutrient series the seedlings grown in the 1.5 atmosphere solution were larger and heavier than those in the 0.5 atmosphere culture. In both seedling harvests of experiment II the lower percentages of dry weight in the cultures of the sodium chloride series, as compared with those of equivalent osmotic concentrations in the other two series, indicated a greater degree of succulence in the high chloride solutions (table 3).

PHYSIOLOGICAL AND CHEMICAL DATA

EXPERIMENT I.-Three plants in each culture were maintained until November 13, a growing period of 51 days.

Except for the 1.5 atmosphere culture in the base nutrient series, in which the plants were taller than those at the 0.5 level, there was a decrease in height at high concentrations of the culture solution in all series. The difference in height between the plants in the 1.5 and 4.5 atmosphere solutions was least in the base nutrient and greatest in the sodium sulphate series.

In all series there was a significant reduction in the diameter of the stem at the high concentrations. This was least in the sodium chloride and greatest in the sodium sulphate series (table 4).

Except for the 1.5 atmosphere culture in the base nutrient series, the total fresh and dry weights of the tops decreased at high concentrations. The fact that the 1.5 atmosphere base nutrient solution promoted greater vegetative growth than that of the 0.5 atmosphere culture suggests that the supply of nutrient salts, especially nitrogen, was more nearly optimum at the 1.5 atmosphere level. All cultures,

TABLE 4

EXPERIMENTS I AND II: AVERAGE DIAMETER OF FIRST INTERNODE OF STEM*

Concentration of solutions (atmospheres)	Series: Base nutrient (mm.) I	Base nutrient (mm.) II	Sodium chloride (mm.) I	Sodium chloride (mm.) II	Sodium sulphate (mm.) I	Sodium sulphate (mm.) II
0.5	10.3	8.0	9.7	8.2	9.0	7.5
1.5	9.9	8.2	9.1	6.8	7.2	6.0
3.0	8.8	7.5	7.5	6.5	4.5	3.2
4.5	7.2	6.7		5.4		2.0
6.0		6.0				

* Average of four stems in each culture.

however, except the high concentrations in the base nutrient series, were supplied with the base nutrient solution at the 0.5 atmosphere level, and no symptoms of nitrogen deficiency were observed.

EXPERIMENT II.—Three plants per culture were maintained until January 2, when two plants from each pot were harvested. The length of the growing period, 48 days, was approximately that of experiment I. The remaining plant in each culture was harvested January 30, 75 days after planting. In all series, at the high levels of concentration the stems were shorter and smaller in diameter than at the control or 1.5 atmosphere levels (table 4).

As in experiment I, the fresh and dry weights of tops decreased with increasing concentration, except in the 1.5 atmosphere base nutrient solution (fig. 1).

In both experiments the percentage of dry matter increased with increasing concentration in the sodium sulphate and base nutrient series and remained practically unchanged in the sodium chloride series. This indicates greater succulence in the high chloride plants than in those of the base nutrient and sodium sulphate series at osmotic concentrations (fig. 2).

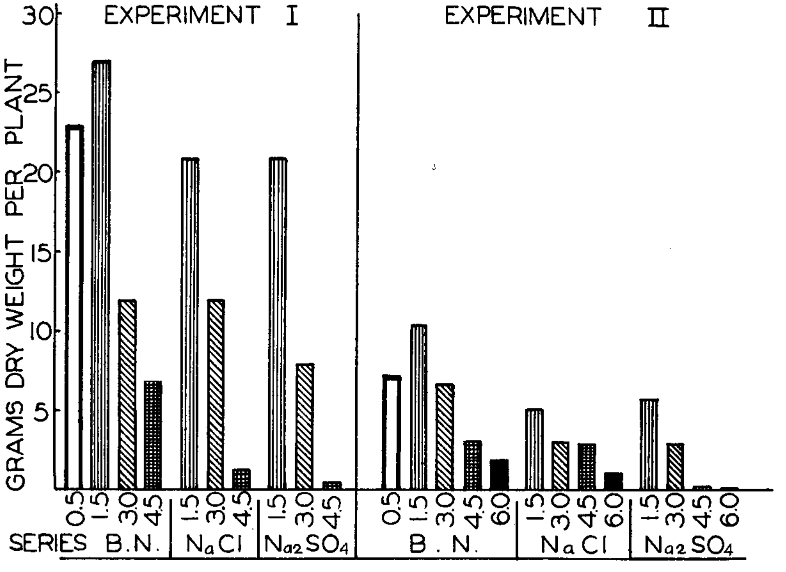

FIG. 1.—Experiments I and II. Average dry weights of tops per plant at harvests of November 10 and January 2. Figures below columns indicate atmospheres osmotic concentration of culture solutions.

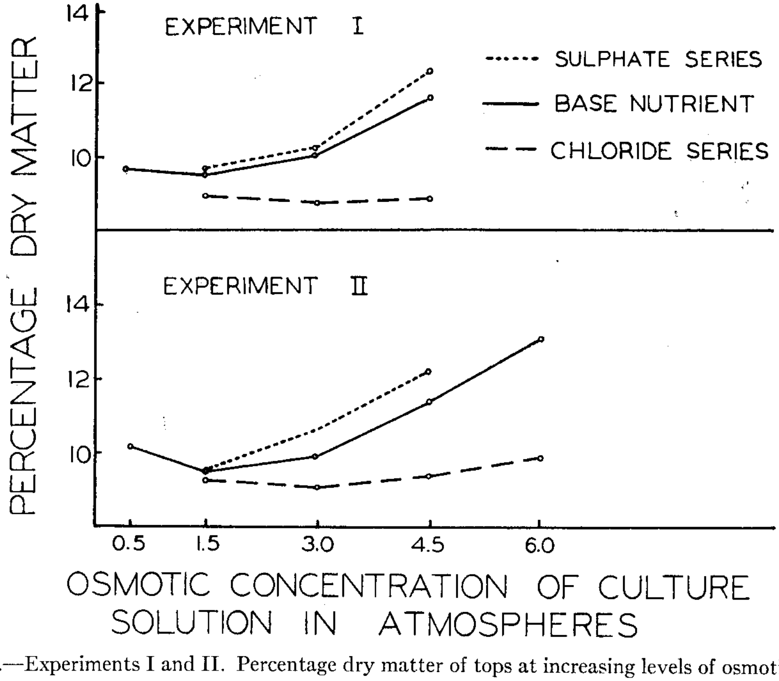

FIG. 2.—Experiments I and II. Percentage dry matter of tops at increasing levels of osmotic concentration in each series.

In corresponding cultures the plants in experiment I were shorter and stockier than those in experiment II and produced greater fresh and dry weights of tops. There was relatively little variation in temperature during the comparable periods of the two experiments, and these differences can probably be ascribed to the light factor, since the solar and sky radiation was 31.5 per cent greater in the first experiment than in the second.

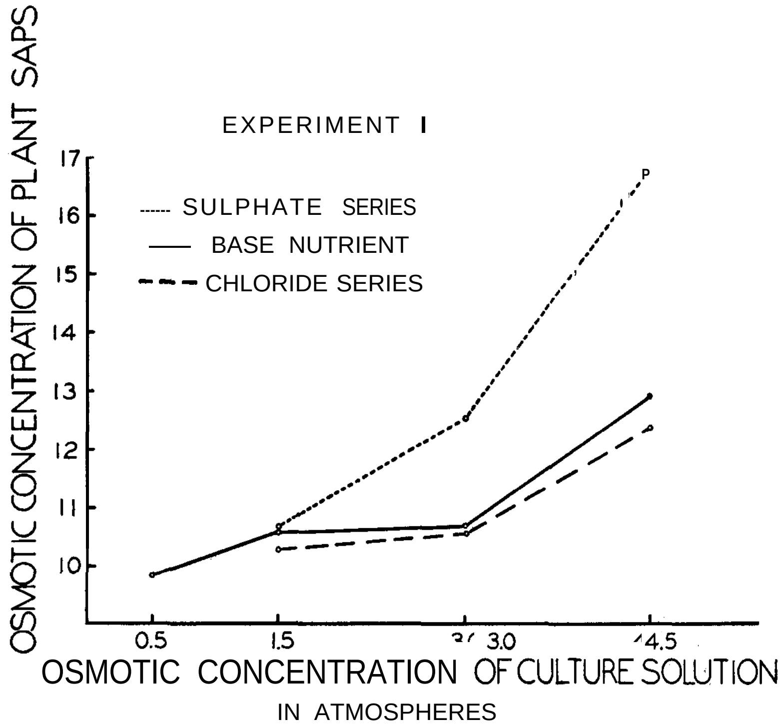

FIG. 3.-Experiment I. Relation between osmotic concentration of plant saps and that of culture solutions.

In both experiments observations on flower-bud formation indicated a definite reduction or retardation in formation in the high sodium chloride solutions and a more pronounced effect in the high sodium sulphate cultures, in which no flower buds were produced up to the time of harvest.

In experiment II all plants except those grown in the 4.5 and 6 atmosphere sodium sulphate solutions had produced evident flower buds at the time of final harvest, but bud formation was retarded at the higher levels in the base nutrient and sodium chloride series. The plants in the 6 atmosphere sodium sulphate solution died before the final harvest. Anthesis was correspondingly delayed at the higher concentrations. The number of inflorescences was greatest in the 1.5 and 3 atmosphere cultures in the base nutrient series and at the 1.5 atmosphere level in the sodium chloride and sodium sulphate series.

The osmotic concentrations of the expressed sap were determined at the conclusion of experiment I. In all series the sap concentration increased with increasing concentration of the culture solution. At isosmotic solution pressures the sap concentrations were highest in the sodium sulphate series. There was close correspondence between the base nutrient and sodium chloride series, the latter having slightly lower sap concentrations (fig. 3).

In experiment II the accumulation of Cl, S, and Na was determined for both harvests of the sodium chloride and sodium sulphate series.[4] Determinations were also made for the 0.5 and 1.5 atmosphere levels of the base nutrient series for purposes of comparison (table 5).

TABLE 5

EXPERIMENT II: BASE NUTRIENT SERIES
ACCUMULATION OF CL, S, AND NA

CONCENTRATION OF SOLUTIONS (ATMOSPHERES)	MILLIEQUIVALENTS PER KILO DRY WEIGHT OF TOPS					
	FIRST HARVEST			SECOND HARVEST		
	CL	S AS SO_4	NA	CL	S AS SO_4	NA
0.5	197	558	147	154	450	127
1.5	112	634	65	151	681	54

In the sodium chloride series there was increasing accumulation of Na and Cl in the tops of the plants with increasing concentration of the culture solution (table 6). In the sodium sulphate series the accumulation of Na increased progressively at the higher concentrations, but there was no marked trend with respect to S intake (table 7).

The results obtained for S accumulation in the sodium sulphate series are not in agreement with unpublished results obtained for tomatoes by EATON[5] at this laboratory. In out-of-doors sand cultures, he found 150, 177, 222, and 299 m.e. of SO_4 per liter in the sap of tomatoes grown in solutions containing 2.68, 50, 160, and 250 m.e. of SO_4 per liter, respectively.

In both sodium series the intake of Na on a dry-weight basis indicates a significant relation to the amount available in the culture solutions. In solutions with

[4] A.O.A.C. methods for Cl and S analyses.

Na analysis: BARBER, H. H., and KOLTHOFF, I. M., A specific reagent for the rapid gravimetric determination of sodium. Jour. Amer. Chem. Soc. 50: 1625-1631. 1928.

[5] F. M. EATON, private communication.

equal concentrations of Na, the accumulation of Na was greater in the sodium sulphate cultures than in those of the corresponding sodium chloride series (fig. **4).** A composite picture of the accumulation of Cl, S, and Na on the basis of dry weight of tops is shown in figure 5.

TABLE 6

EXPERIMENT II: SODIUM CHLORIDE SERIES
ACCUMULATION OF CL AND NA

CONCENTRATION OF SOLUTIONS (ATMOSPHERES)	MILLIEQUIVALENTS PER KILO DRY WEIGHT OF TOPS			
	FIRST HARVEST		FINAL HARVEST	
	CL	NA	CL	NA
1.5	880	653	754	563
3.0	1090	1320	911	934
4.5	1480	1780	1340	1445
6.0	1800	2285	1520	1645

TABLE 7

EXPERIMENT II: SODIUM SULPHATE SERIES
ACCUMULATION OF S AND NA

CONCENTRATION OF SOLUTIONS (ATMOSPHERES)	MILLIEQUIVALENTS PER KILO DRY WEIGHT OF TOPS			
	FIRST HARVEST		FINAL HARVEST	
	S AS SO_4	NA	S AS SO_4	NA
1.5	828	1090	694	709
3.0	749	2180	506	1585
4.5	1080	25256565	574	2275

ANATOMICAL RESPONSES

At harvest all plants were sampled to obtain representative material for anatomical analysis. In selecting material, care was exercised to establish a definite reference point, so that the structures examined would be comparable. The stem segments were selected from the median portion of the basal internode immediately above the cotyledonary node. Mature leaves were selected, and a rectangular segment of the terminal leaflet, oriented so as to include a portion of the midvein, was taken at a median point in the lamina.

The material was fixed with Navashin's solution, dehydrated in an ethyl-tertiary butyl alcohol series, and infiltrated with a paraffin-beeswax-rubber mixture. Immediately after placing the material in the fixative, air was evacuated from the tissues with a vacuum pump. Sections were cut at 15 μ and stained with a modified Flemming's triple stain. The photomicrographs of all series were taken at the same magnification.

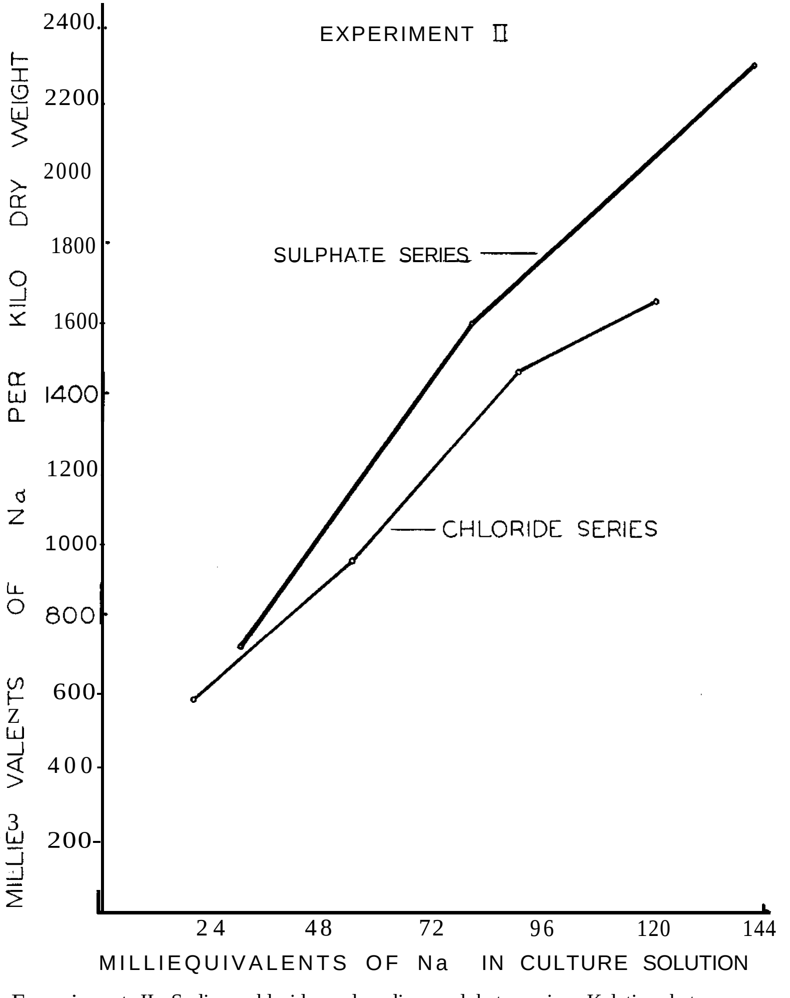

FIG. 4.-Experiment II. Sodium chloride and sodium sulphate series. Kclation between concentration of sodium in culture solution and accumulation of sodium in tops.

STEM ANALYSIS.-The tomato stem has a dissected siphonostele consisting of three or four bicollateral bundles which at first are separated by broad medullary rays. As the stem matures the fascicular and interfascicular cambiums form a continuous cylinder, and a solid zone of secondary vascular tissue is produced. The mechanical tissues consist of a band of collenchyma lying centrad to a narrow zone of chlorenchyma, and the outer and inner pericyclic fibers. A very small number of inner pericyclic fibers was differentiated (5).

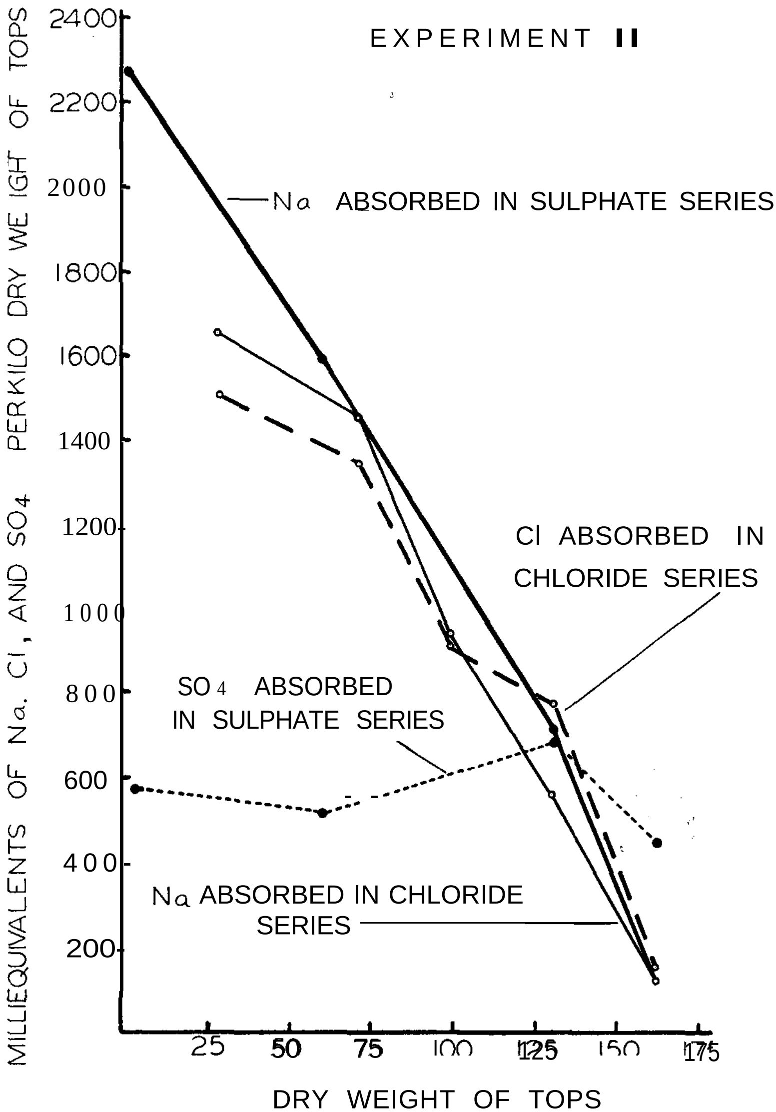

FIG. 8.—Experiment II. Sodium chloride and sodium sulphate series. Relation between accumulation of sodium, chloride, and sulphate ions and dry weight of tops.

With few exceptions the anatomical responses in corresponding cultures of the two experiments agreed with respect to their general character and differed only in degree. For this reason the results obtained in experiments I and II are discussed jointly for each series. Additional observations are included for the 6 atmosphere level in experiment II.

The smaller diameters of stems grown in solutions of high salt concentration were the result of differential reduction in their tissue systems. In general, the reduction of the vascular tissues was greater than that of the parenchymatous tis-

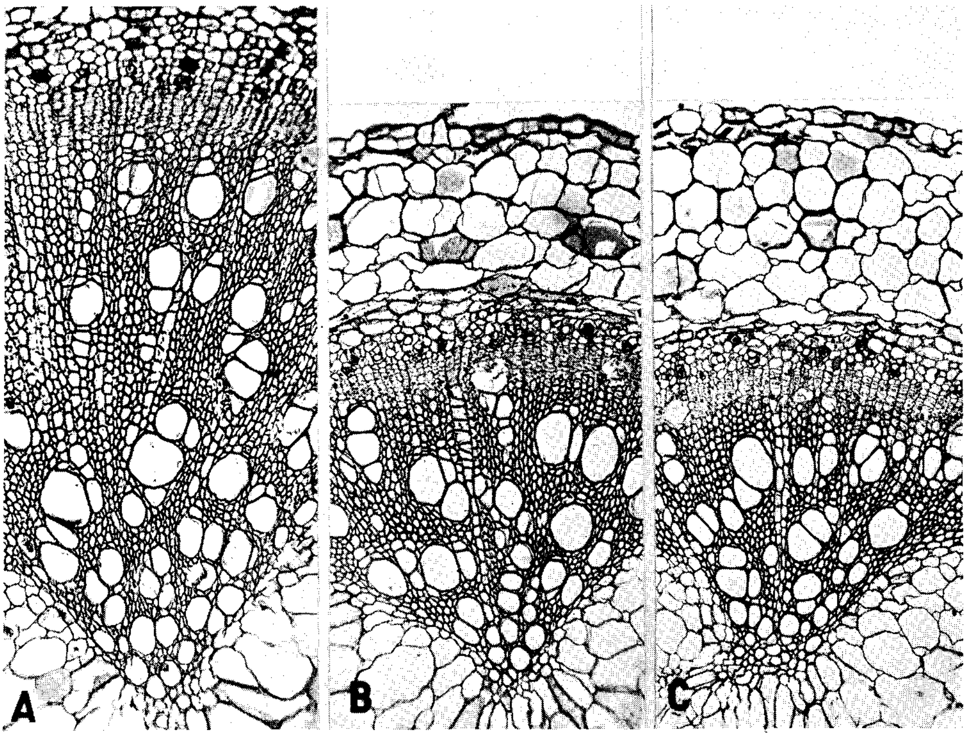

FIG. 6.-Experiment II. Base nutrient series. Transections of basal internodes showing progressive growth inhibition with increasing concentration of culture solution. Sector of largest vascular bundle shown in each case. Osmotic concentrations in atmospheres: A, 0.5; *B, 4.5;* and C, 6.

sues of the cortex and pith. Measurements were taken of the width of cortex, radial dimension of the largest bundle, and width of the fascicular xylem, outer phloem, and interfascicular vascular tissue. The largest bundle in each transection was measured, as it was determined that its size constituted a reliable index of the total amount of fascicular tissue.

BASE NUTRIENT SERIES.--In both experiments there was a marked decrease in the amount of secondary vascular tissue at the high concentrations as compared with the 0.5 and 1.5 atmosphere cultures (fig. 6). Development of interfascicular vascular tissue followed the same general trend as that of the bundles. The reduction in the secondary (outer) phloem was proportionately greater than that of the xylem. The inner phloem was not measured, since little differential growth response occurred in the primary tissues (table 8).

TABLE 8

EXPERIMENTS I AND II: GROWTH MEASUREMENTS OF BASAL STEM* (IN MICRONS)

CONCENTRATION OF SOLUTIONS (ATMOSPHERES)	LARGEST BUNDLE I	LARGEST BUNDLE II	XYLEM I	XYLEM II	OUTER PHLOEM I	OUTER PHLOEM II	INTERFASCICULAR VASCULAR TISSUE I	INTERFASCICULAR VASCULAR TISSUE II
	BASE NUTRIENT SERIES							
0.5	1910	1550	1550	1190	284	334	357	471
1.5	1900	1380	1610	1130	246	215	405	319
3.0	1530	1140	1250	968	191	152	349	258
4.5	1260	872	1070	723	136	114	243	212
6.0	......	633	....	532	....	101	....	117
	SODIUM CHLORIDE SERIES							
1.5	1900	1250	1610	1040	232	171	440	266
3.0	1600	942	1310	794	205	129	326	180
4.5	912	912	729	771	106	110	167	167
6.0	......	765	....	627	....	106	....	129
	SODIUM SULPHATE SERIES							
1.5	1650	1160	1350	934	223	197	395	238
3.0	1410	881	1120	714	182	136	326	238
4.5	737	471	592	380	98	60	174	115
6.0	......	288	....	259	....	26	....	80

*Radial dimensions, averages of four stems in each culture.

The rate of cambial activity was slightly higher at the 1.5 atmosphere level than at the 0.5, but was depressed at the high concentrations. As compared with the 1.5 atmosphere culture, the reduction in cambial rate at the 4.5 and 6 atmosphere levels was 25 per cent and 39 per cent, respectively. The total growth depression was a result of reduction in cell size as well as in number of cells formed. This cellular response was indicated by the decrease in the size of the xylem vessels at the high concentrations (table 9).

There was a marked decrease in the total amount of parenchyma in plants grown in the more concentrated culture solutions. With few exceptions, the percentage of medullary tissue increased with increasing osmotic concentration of the culture, while there was little change in the percentage of cortical tissue (table 10).

The differences in the areas of pith may be attributed mainly to variation in the size of the cells, which were larger in stems grown at the lower levels of concentration. The same condition prevailed in the cortex, but in some cases the tangential enlargement of the cortical cells was accompanied by radial divisions.

The collenchymatous cells of plants grown in the 4.5 and 6 atmosphere solutions were smaller than those in the less concentrated cultures, and the thickening

TABLE 9

EXPERIMENTS I AND II: ALL SERIES. MAXIMUM RADIAL DIMENSIONS OF SECONDARY XYLEMVESSELS(IN MICRONS)

Concentration of solutions (atmospheres)	Base nutrient		Sodium chloride		Sodium sulphate	
	I	II	I	II	I	II
0.5..	150	126	....	....	....	.
1.5...........	152	139	141	132	133	126
3.0...	126	123	'3.5	114	99	89
4.5.....	117	109	108	92	69	45
6.0.................		91		85		29

of the walls at the angles of the cells was more pronounced. The pericyclic fibers in plants grown at the higher osmotic concentrations were smaller in caliber and their secondary walls were proportionately thicker in comparison with the lumina of the cells.

SODIUM CHLORIDE SERIES.--As in the base nutrient series, there were significant reductions in the diameters of the stems grown in culture solutions with high osmotic concentrations as compared with those grown at the 1.5 atmosphere and control (0.5 atmosphere) levels (table 4).

The effect of high salt concentration on the development of secondary xylem and phloem was most pronounced in the more vigorously growing plants of experiment I; but in both experiments there was marked reduction in the amount of secondary fascicular xylem and phloem and in the development of interfascicular vascular tissue (fig. 7 ; table 8) .

In this series, using the 1.5 atmosphere culture as a basis of comparison, the cambial activity was reduced approximately 25 per cent in the 4.5 and 34 per cent in the 6 atmosphere solutions. The effect of high salt concentration on the secondary xylem tissue was evidenced also by the reduction in the size of the vessels and increase in the thickness of their walls (table 9). The reduction in the amount of cortical and medullary parenchyma produced at the high levels of salt concentration was proportional to the reduction in the size of the stem. There was little variation in the percentage of the total area comprising the cortical and medullary parenchyma (table 10). The differentiation of collenchyma and pericyclic fibers was similar to that in the base nutrient series, but the thickness of the walls was more pronounced at the higher levels.

TABLE 10

EXPERIMENTS I AND II: TRANSECTIONAL AREAS OF STEM AT FIRST INTERNODE*

CONCENTRATION OF SOLUTIONS (ATMOSPHERES)	TOTAL AREA (SQ. MM.)		PERCENTAGE OF TOTAL AREA			
			CORTEX		PITH	
	I	II	I	II	I	II
	BASE NUTRIENT SERIES					
0.5	84	50	19	28	46	35
1.5	76	53	16	21	45	46
3.0	61	44	14	20	50	50
4.5	41	35	16	20	50	53
6.0		28		21		57
	SODIUM CHLORIDE SERIES					
1.5	74	50	18	22	44	52
3.0	63	36	18	21	47	53
4.5	44	33	17	21	58	51
6.0	....	23	....	28	....	46
	SODIUM SULPHATE SERIES					
1.5	63	44	19	20	45	50
3.0	41	28	21	26	41	45
4.5	15	8	25	43	43	33
6.0	....	3	....	64	....	16

* Averages of four stems in each culture.

SODIUM SULPHATE SERIES.—The growth responses followed the same general pattern observed in the base nutrient and sodium chloride series, but the amount of growth depression was greater in this series at corresponding osmotic concentrations (table 4). At equal osmotic concentrations the total amount of vascular tissue was less in the sodium sulphate series than in the sodium chloride series (fig. 8). The reduction in the amount of secondary phloem was greater than that of secondary xylem, and this difference was more pronounced than in the base nutrient series. In the sodium sulphate series the amount of growth reduction was much greater between the 3 and 4.5 atmosphere levels than between the 1.5 and 3 atmosphere cultures (table 8).

The rate of cambial activity was much depressed at the 4.5 atmosphere level, being approximately 40 per cent less than that at the 1.5. In the 6 atmosphere culture there was no evidence of interfascicular activity in some of the stems, and the

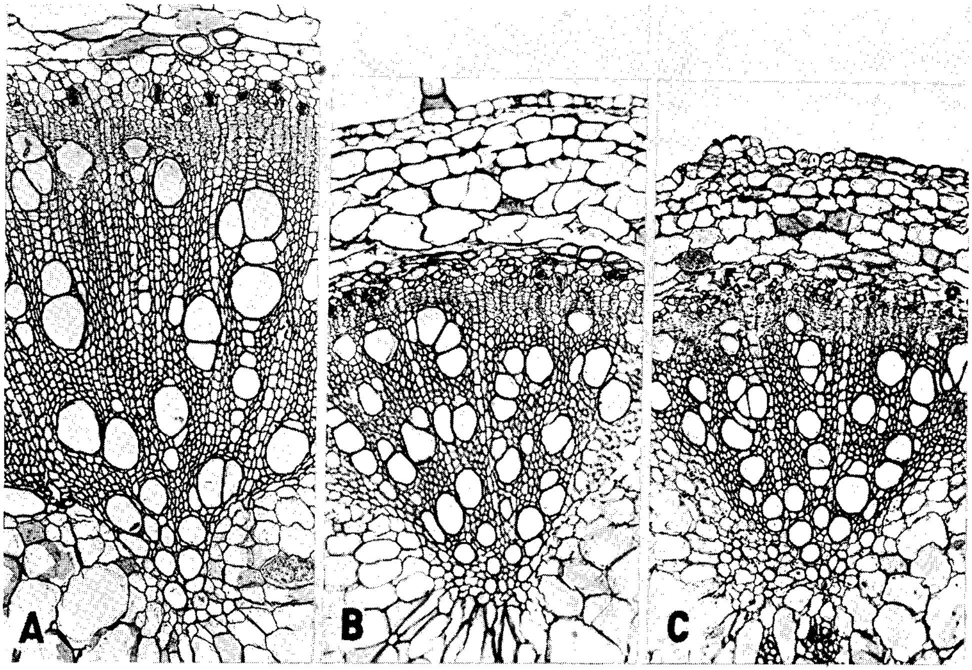

FIG. 7.-Experiment II. Sodium chloride series. Transections of basal internodes showing progressive growth inhibition with increasing concentration of culture solution. Sector of largest vascular bundle shown in each case. Osmotic concentrations in atmospheres: *A*, 1.5; *B*, 4.5; and C, 6.

rate of activity of the fascicular cambium was about 60 per cent less than in the 1.5 atmosphere culture. As in the preceding series, there was a reduction in the size of the secondary xylem vessels and a relative increase in the thickness of the cell walls at the high concentrations (table 9).

In experiment I the proportion of cortical and medullary tissues to total transectional area increased slightly with increasing concentration, except at one level. In experiment II the percentage of cortical parenchyma increased markedly, while the medullary tissue decreased. The percentage of the total area comprised of parenchymatous tissue increased at the high concentrations, although the absolute amounts were much decreased (table 10).

There was pronounced accumulation of starch in the parenchymatous cells at the high concentrations. At the 4.5 and 6 atmosphere levels the cells of the endodermis and the pith cells adjacent to the vascular bundles were filled with starch grains. The cortical parenchyma and ray cells also contained more starch than at equivalent concentrations in either of the other series (fig. 8). At the high concentrations the cells of the collenchyma and the pericyclic fibers were smaller and the walls thicker in proportion to the size of the lumina.

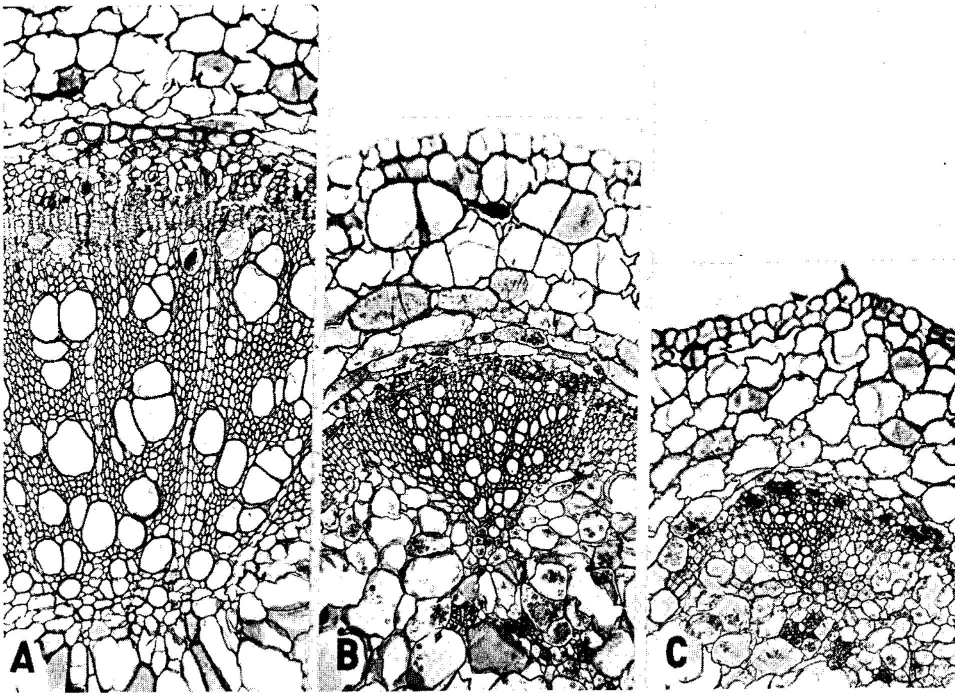

FIG. 8.--Experiment II. Sodium sulphate series. Transections of basal internodes showing progressive growth inhibition with increasing concentration of culture solution. Pronounced accumulation of starch'indicated in B and C. Largest bundle shown in each case. Osmotic concentrations in atmospheres: A, 1.5; *B, 4.5;* and C, 6.

LEAF ANALYSIS

The blade of the leaflet is commonly thin, and the mesophyll consists of a single row of palisade cells and a spongy parenchyma of four or five layers of loosely arranged cells. The main vein projects prominently on the lower surface (fig. 9), and its bicollateral bundle is reinforced by abaxial and adaxial strands of collenchyma (5).

Transections of leaves were examined to determine the growth responses of the vascular tissue of the main vein and the tissues of the blade. The leaves were selected from the final harvest of experiment I and the first harvest of experiment II. Leaves from the final harvest of experiment II were also examined, but these were not strictly comparable with those of the other two harvests, since they were 4 weeks older and had been subjected to less favorable environmental conditions during the additional period of growth. In all series, leaves from the 1.5 and 4.5 atmosphere cultures were examined, and additional studies were made from the 0.5 atmosphere culture in the base nutrient series and the 6 atmosphere cultures of experiment II.

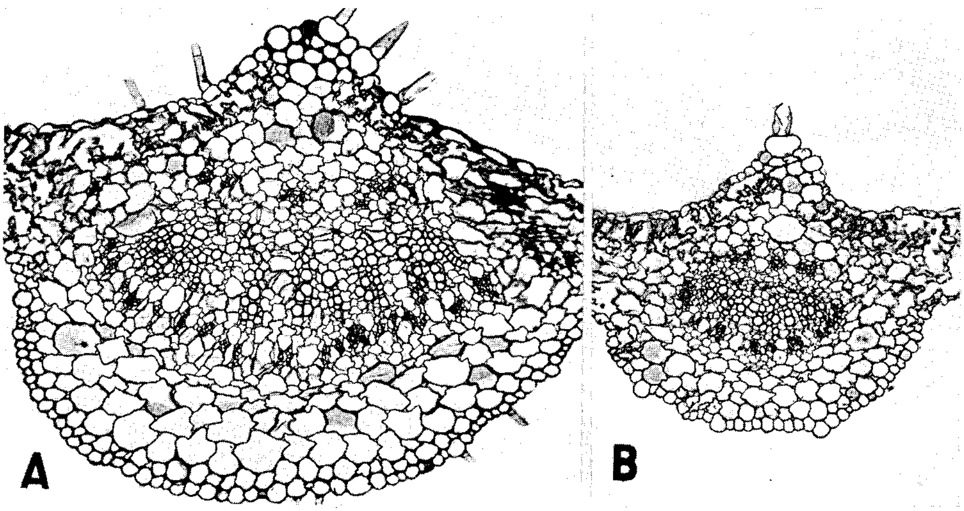

FIG. 9.--Experiment II. Sodium sulphatc series. Transections of midribs of terminal leaflets showing growth inhibition at higher concentration of culture solution. Atmospheres osmotic concentration: A, 1.5; B, 4.5.

Representative leaves selected from the median portion of the plant axis were used for biometrical analysis. The height of the vascular bundle of the midrib of the terminal leaflet was used as an index of the amount of vascular tissue supplying the leaves. It provided a suitable basis of comparison with the radial dimensions of the vascular bundles of the stem. The average thickness of the lamina, the height of the palisade layer, and the thickness of the spongy parenchyma were also determined.

BASE NUTRIENT SERIES.-In this series the height of the vascular bundle of the main vein decreased at each successively higher concentration of the culture solution, resembling the growth depression of the bundles of the stem in this regard. In experiment II comparison of leaves from the 1.5 and 4.5 atmosphere cultures indicated a significant reduction in the thickness of the leaf of the latter, while in experiment I an increase occurred (table II).

SODIUM CHLORIDE SERIES.—In both experiments there was a decrease in the vascular tissue of the midvein at the 4.5 as compared with the 1.5 atmosphere culture, and further growth depression was indicated at the 6 atmosphere level in experiment II. There was a significant increase in the thickness of the blade at the higher concentrations. The leaves were about 9 per cent thicker in the 4.5

TABLE 11

EXPERIMENTS I AND II: LEAF MEASUREMENTS (IN MICRONS)

Concentration of solutions (atmospheres)	Main bundle height I	Main bundle height II	Lamina thickness I	Lamina thickness II	Palisade height I	Palisade height II	Spongy parenchyma thickness I	Spongy parenchyma thickness II
				BASE NUTRIENT SERIES				
0.5	....	273	....	187	....	70		92
1.5	360	237	158	177	61	57	73	84
4.5	253	223	174	151	61	56	87	72
6.0	....	174	....	139		$3		64
				SODIUM CHLORIDE SERIES				
1.5	314	246	176	156	67	58	87	70
4.5	228	190	192	170	71	68	101	74
6.0	....	'77	....	181	....	76	....	82
				SODIUM SULPHATE SERIES				
1.5	[illegible]	145	[illegible]	175	[illegible]	[illegible]	116	73 85
6.0		148		192		69	....	93

than in the 1.5 atmosphere cultures; and in experiment II those from the 6 atmosphere culture were 16 per cent thicker than leaves grown at 1.5 (table 11). Leaves from the final harvest of experiment II did not exhibit growth responses similar to those just recorded. The leaves grown at the 4.5 and 6 atmosphere levels were much thicker than those of the 1.5, but the leaves of the plants grown at 6 atmospheres were thinner than those of the 4.5.

SODIUM SULPHATE SERIES.—In both experiments there was a pronounced decrease in the size of the main bundle at the high concentrations (fig. 9). As compared with the 1.5 atmosphere culture, the growth at 6 was depressed about 45 per cent in the sodium sulphate series and 28 per cent in the sodium chloride.

There were significant increases in the thickness of the leaves at the 4.5 and 6 atmosphere levels, ranging from 9 per cent to 30 per cent. At the 4.5 atmosphere level the leaves of the sodium sulphate series were slightly thicker than those of the sodium chloride culture (table II). At the high levels of salt concentration the relative density of the mesophyll tissues in the sodium chloride and sodium sulphate series appeared to be correlated with the degree of succulence. The lower percentage of dry matter in the tops of the sodium chloride plants as compared with those of the sodium sulphate and base nutrient series at equivalent concentration levels has been noted. In the leaves of plants grown in the high sodium sulphate cultures the palisade layer was much more compactly organized than in the leaves of the corresponding sodium chloride cultures (fig. 10).

Discussion

The significance of high concentrations of the soil solution in limiting the intake of water and sdluble salts has been pointed out by MAXIMOV (8). BREAZEALE (1), growing wheat in solutions in which the concentration of nutrient salts ranged from 15 to 1550 p.p.m., concluded that the optimum concentration was approximately 155 p.p.m. SHIVE (12), using a three-salt solution, obtained best results at 1.75 atmosphere osmotic concentration, poorest ones at 0.1 atmosphere, and intermediate effects at 4. HOAGLAND (6) has defined optimum total concentration of a nutrient solution as "the least concentration giving a yield equal to any higher concentration." On that basis he found the optimum concentration to be 0.6 atmosphere and inhibitive concentrations at 2-2.5 atmospheres. TRELEASE (13) obtained optimum yields at 1.6 atmosphere, and found decreasing dry weight of tops with increasing concentration. NIGHTINGALE and FARNHAM (11) obtained best results with sweet peas at 0.5-1 atmosphere osmotic concentration.

In the present experiments with nutrient cultures at osmotic concentrations of 0.5, 1.5, 3, 4.5, and 6 atmospheres, maximum vegetative growth was obtained at 1.5 And a pronounced growth depression was noted at higher concentrations. No cultures were maintained at 1 atmosphere, and it is possible that for the solution used the optimum concentration as defined by HOAGLAND may lie somewhere between 0.5 and 1.5 atmosphere.

In cases where the high osmotic concentrations were obtained by the addition of sodium chloride or sodium sulphate to the base nutrient solution (0.5 atmosphere concentration), the growth depression at isosmotic concentrations was greater than in the base nutrient cultures. Comparisons of fresh and dry weights, gross morphology, and detailed anatomy indicated that the plants in the high sodium sulphate cultures were smaller than those in the corresponding cultures in the sodium chloride series. This suggests that there is an ionic influence which operates in addition to the effect of total salt concentration. EATON (3) has indicated

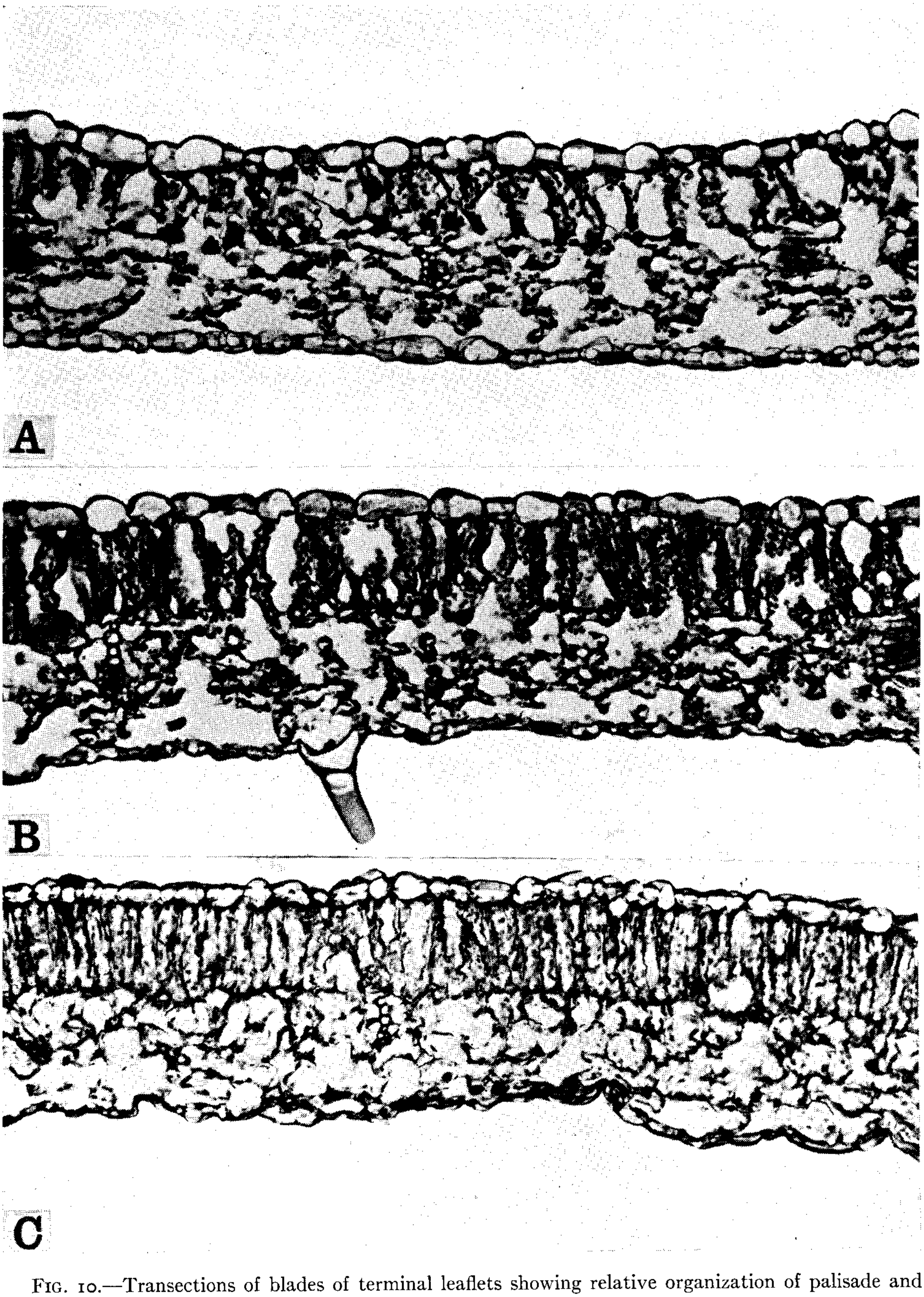

FIG. 10.—Transections of blades of terminal leaflets showing relative organization of palisade and spongy parenchyma: *A*, base nutrient culture, 1.5 atmosphere; *B*, sodium chloride culture, 4.5 atmospheres; *C*, sodium sulphate culture, 6 atmospheres.

a tendency for sulphate to be about half as toxic as chloride on a milliequivalent basis, but notes that "the tomato is out of line" in this regard.

In the sodium chloride series the percentage of dry matter increased very slightly or not at all with increasing concentration, while in the base nutrient series--and to a greater degree in the sodium sulphate series-there were marked increases in the percentage of dry matter, indicating a greater degree of succulence in the high chloride series.

The succulence of the leaves in the sodium chloride series corresponded with that observed by WUHRMANN (16), who found that the thickness and degree of succulence of the leaves of *Lepidium sativum* and *Nicotiana* could be modified by the addition of sodium chloride to nutrient solutions. VAN EIJK (14) investigated the effect of sodium chloride on the halophyte Salicornia *herbacea* and found optimum development with a 4/12 molar solution containing 2 per cent sodium chloride. He attributed the growth responses to the chloride ion rather than to the osmotic value of the solution or the concentration of the sodium ion, and concluded that cations do not affect succulence and that it is not induced by sulphate or nitrate ions. It seems possible, however, that the sodium ion may have an inhibitory effect on growth. At equal osmotic concentrations the solutions in the sodium sulphate series had a higher concentration of sodium than the sodium chloride cultures, and the accumulation of sodium by the plants showed a close relationship to the amount of sodium present in the solution, irrespective of the anion.

Growth depression may be the result of environmental factors which restrict the size of cells by accelerating their rate of maturation, or which inhibit the rate of activity of primary or secondary meristems. In the stems examined the number of primary cells at any given transectional level did not appear to be materially reduced by high salt concentration. The cells of the fundamental parenchyma were smaller, but the elements of the primary vascular tissue did not exhibit significant variation in size. The cells derived from the secondary meristems, especially the xylem elements, were smaller in size at the high concentrations.

In all series there was less cambial activity in plants grown in culture solutions with high osmotic concentrations than in those at the 1.5 atmosphere levels. Comparisons of cambial rate indicated that, at isosmotic concentrations, the rate was greatest in the base nutrient series, least in the sodium sulphate, and intermediate in the sodium chloride series. Between the 1.5 and 4.5 atmosphere levels the reduction in cambial rate ranged from 25 per cent to 42 per cent.

Starch accumulation may result from a number of factors. NIGHTINGALE (9) and FOSTER and TATMAN (4) have indicated the influence of temperature in this regard, and the latter have also called attention to the effects of variation in soil moisture. A number of investigators have shown that the carbohydrate metabolism of the tomato plant is influenced by the supply of nutrient constituents, including ammonium, nitrate nitrogen, and potassium (7, 10, 15). Although no quantitative data were obtained, pronounced accumulation of starch in the storage parenchyma of the basal portion of the stem appeared to be correlated with high salt concentration. This was most evident in the high sodium sulphate cultures, in which the medullary, ray, and cortical parenchyma and the endodermal cells contained large reserves of starch.

Summary

Tomato plants were grown in three series of culture solutions: (a) a base nutrient series consisting of cultures adjusted to 0.5, 1.5, 3, 4.5, and 6 atmospheres osmotic concentration; (b) a sodium chloride series consisting of cultures containing base nutrient (0.5 atmosphere) plus sodium chloride to adjust the concentrations to 1.5, 3, 4.5, and 6 atmospheres osmotic concentration, respectively; and (c) a sodium sulphate series set up as in (b) but with sodium sulphate as the added salt instead of sodium chloride.

1. The concentration of the culture solution exerts an important influence on growth. In all series the growth of the plants as measured by height, diameter of stem, and dry weight was less at the high concentrations than at the control or 1.5 atmosphere levels. The greatest differences occurred in the sodium sulphate and the smallest in the base nutrient series.

2. The smaller diameters of stems at the high levels of salt concentration were correlated with significant differential reductions in the tissue systems. In general, the reduction of the vascular tissues, on the basis of percentage of total area, was greater than that of the parenchymatous tissue of the cortex and pith.

3. The reduction in the amount of vascular tissue at the high levels of salt concentration was related to more rapid maturation and consequent reduction in the size of the cells. The secondary xylem vessels were smaller in diameter at the high concentrations in all series, and the walls were thicker in proportion to the lumina of the vessel segments.

4. In the base nutrient and sodium sulphate series the proportion of secondary phloem to xylem was decreased at the high levels of salt concentration. In the sodium chloride series the proportionate amounts of secondary xylem and phloem remained approximately the same at all levels.

5. In all series the cells of the mechanical tissues were smaller in caliber and thicker walled in plants grown at high osmotic concentrations.

6. Cambial activity was inhibited by high concentrations of salts in all series. At isosmotic concentrations the cambial rate was greatest in the base nutrient and least in the sodium sulphate cultures.

7. The leaflets of plants grown at the high salt concentrations were significantly thicker in the sodium chloride and sodium sulphate series than in the base nutrient cultures.

8. The leaves of plants grown in high sodium chloride solutions were more succulent than those of the base nutrient and sodium sulphate series. This was correlated with the loose arrangement of the tissues of the mesophyll. In contrast, the palisade tissue in the leaves of plants grown in high sodium sulphate cultures was very compact.

9. The greater succulence of plants grown in high sodium chloride solutions was also indicated by the percentage of dry matter of tops. This remained essentially constant at all levels of concentration in the sodium chloride series, while it increased at the high concentrations in the base nutrient and sodium sulphate cultures.

10. Flower bud formation was retarded and probably reduced on plants grown in high sodium chloride solutions, and anthesis was delayed. There was a more pronounced effect in the high sodium sulphate cultures at the 4.5 atmosphere level, and no evident flower buds had developed up to the time of harvest.

11. High starch accumulation in the parenchymatous tissues of the basal stem was apparently correlated with high salt concentration in the culture solutions. This was most pronounced in the sodium sulphate cultures at 4.5 and 6 atmospheres osmotic concentration, but was also marked in the stems of plants grown at. high sodium chloride levels.

12. Osmotic concentration of the sap increased in all series with increasing concentration of the culture solution.

13. With increasing concentration of the culture solutions there was increased accumulation of sodium and chloride ions in the tops of plants in the sodium chloride series. In the sodium sulphate series accumulation of the sodium ion increased as the concentration of the culture solution increased, but there was less correlation between the accumulation of the sulphate ion and the concentration of sulphate in the solutions.

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LITERATURE CITED

1. Breazeale, J. P., Effect of the concentration of the nutrient solution upon wheat cultures. Science 22:146-149. 1905.
2. Eaton, F. M., Plant culture equipment. Plant Physiol. 16: 385-392. 1941.
3. ——— The toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. In Press.

4. FOSTER, A. C., and TATMAN, E. C., Influence of certain environmental conditions on congestion of starch in tomato plant stems. Jour. Agr. Res. 56:869-881. 1938.
5. HAYWARD, H. E., The structure of economic plants. New York. 1938.
6. HOAGLAND, D. R., Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. Jour. Agr. Res. 18:73-117. 1919.
7. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction, with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
8. MAXIMOV, N. A., The plant in relation to water. London. 1929.
9. NIGHTINGALE, G. T., Effects of temperature on metabolism in tomato. BOT. GAZ. 95:35-58. 1933.
10. NIGHTINGALE, G. T., ROBBINS, W. R., and SCHERMERHORN, L. G., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in the roots, stems, and *leaves*. *New* Jersey Agr. Exp. Sta. Bull. 461. 1928.
11. NIGHTINGALE, G. T., and FARNHAM, R. B., Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. BOT. GAZ. 97:477-517. 1936.
12. SHIVE, J. W., A study of physiological balance in nutrient media. Physiol. Res. 1:327-397. 1915.
13. TRELEASE, S. F., The relation of the concentration of the nutrient solution to the growth of young wheat plants in water cultures. Johns Hopkins Univ. Circ. 3:225-227. 1917.
14. VAN EIJK, M., Analyse der Wirkung des NaCl auf die Entwicklung, Sukkulenz und Transpiration bei Salicornia *herbacea,* sowie Untersuchungen uber den Einfluss der Salzaufnahme auf die Wurzelatmung bei *Aster tripoliurn.* Rec. Trav. Bot. Neerland. 36: 559-657. 1939.
15. WALL, M. E., The role of potassium in plants. II. Effect of varying amounts of potassium on the growth status and metabolism of tomato plants. Soil Sci. 49:315-331. 1940.
16. WUHRMANN, K., Die Ionenaufnahme der Pflanzen und ihre morphogenetische Wirkung. Eidg. Tech. Hochschule. Zurich. 193.5.