UPTAKE OF LYSOZYME BY *LYCOPERSICON ESCULENTUM* ROOTS

by

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STATEMENT BY AUTHOR

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

An attempt was made to determine whether lysozyme, added to the solution around roots, was absorbed by tomato roots and translocated upward in the xylem. The tops of tomato plants were excised, and the exudate collected from the stumps of the root system was analyzed for the presence of lysozyme. A substrate suspension prepared from killed cells of Micrococcus lysodeikticus was mixed with the exudate. The optical density reduction of the mixture was measured with a spectrophotometer.

Lysozyme was supplied to the roots at a concentration of 0.1 mg/ml in distilled water, modified Hoagland's solution, or 0.067M Sorenson's phosphate buffer at pH's of 5, 6, 7, 8, and 9. Lysozyme was not detected in root pressure exudate from any of these experiments. When 2 atm pressure was applied to a 0.6 mg/ml lysozyme solution around the roots, however, the solution was forced through the roots, and lysozyme was detected in the exudate.

Higher concentrations of lysozyme were found in exudate from 10 to 11-week old roots than from 5 or 7-week old roots. The concentration of lysozyme in exudate from the younger roots increased when dimethyl sulfoxide was added to the solution around the roots.

INTRODUCTION

Due to their large size, proteins generally were thought not to be taken up in plant roots. There have been several reports, however, indicating that proteins are taken up by roots. Whether the unaltered protein reaches the xylem and is translocated to the foliar portion of the plant is not clear from these reports, however.

Kraus, von Portheim, and Yamanouchi (1907) reported the entry of animal antibodies into the roots of uninjured bean plants. Moritz and vom Berg (1932), by using an anaphylactic shock technique, discovered that ovalbumin was absorbed and translocated by Vicia. More recently, Kaufman and Das (1955), Bracket (1954), Bhide and Brachet (1960), and Alfert and Das (1962) showed by mitotic abnormalities that RNAse was taken up by plant roots.

Through the use of autoradiograms, McLaren, Jensen, and Jacobson (1960) demonstrated that C-14 labeled and tritiated lysozyme, hemaglobin, and ribonuclease could be found within cells of the epidermis, cortex, and xylem of excised barley roots. The uptake of C-14 labeled lysozyme, hemaglobin, beta lactoglobin, and ovalbumin into sterile tomato root cultures was studied by Ulrich and McLaren (1965). When the plant roots were supplied with C-14 labeled proteins and then subjected to mechanical damage or temporary wilting, autoradiography of the foliar portions of the plant revealed the presence of the proteins and/or protein dehydration products.

Bradfute and McLaren (1964) ascertained that metabolic inhibitors such as cyanide, DNP, fluoride, and carbon monoxide did not inhibit lysozyme uptake, but mechanical injury did improve uptake. Decreased temperature, decreased pH, and increased ionic strength of the buffer caused a decrease in absorption both in live and ether-killed roots.

It was demonstrated by Jacob et al (1964) that dimethyl sulfoxide (DMSO) enhanced the passage of insulin into the bloodstream of dogs. In similar studies Jacob et al (1964) demonstrated that DMSO enhanced the passage of heparin, serum sulfadiazine, salicylate, Evans Blue dye, and aminophylline across dog bladders. Keil (1965) has shown that sprays of Terramycin and DMSO gave better control of peach bacterial leaf spot than Terramycin alone. These studies suggest that DMSO may cause modification in the cell membrane and permit enhanced uptake of molecules much larger than inorganic ions. It also may act as a vehicle to carry organic compounds "piggy-back" through plant tissues.

This study was undertaken to determine if a foreign protein supplied to the solution around the roots could be detected in the root exudate of detopped plants having intact roots systems. If functional lysozyme could be detected this would indicate that uptake, movement across the cortex, and entrainment in the transpirational stream was taking place. To test this hypothesis experiments were performed to examine the possibility of active movement across the root and to study the effect of simulated transpiration upon uptake by pressure induction of water flow through the root systems. DMSO was used in this study to determine if it would enhance protein uptake in the roots.

METHODS AND MATERIALS

The protein used in this study was lysozyme three times crystallized from egg albumin (Calbiochem lot number 54106). According to Jolles (1960) this enzyme has a molecular weight of 14,500 and is composed of about 130 amino acid residues. It has an isoelectric point between 10.5 and 11 and is stable in acid pH's at 100°C but becomes unstable in alkaline pH's at high temperatures. Native lysozyme is not digested by trypsin, chymotrypsin, or papain, but it is digested by pepsin. Lysozyme is active over a pH range of from 5 to 10.

A bioassay developed by Smolelis and Hartsell (1949) was used to detect the presence of lysozyme. An enzymatic bioassay was particularly advantageous in this study because it not only provided a means of detection, but it also provided assurance that the protein had retained its native configuration at least with respect to active enzymatic sites.

The substrate used for the bioassay consisted of killed Micrococcus lysodeikticus cells. The stock culture of M. lysodeikticus was obtained from the American Type Culture Collection number 4689. Upon receipt, the stock culture was planted onto veal infusion broth medium. After 48 hours growth at 37°C (Smolelis and Hartsell, 1949) a loop of broth medium was serially streaked onto a petri plate of veal infusion agar medium. This plate was placed in a 37°C incubation chamber for 24 hours after which the plate was examined. Visual inspection showed that the plate was composed entirely of small yellow pigmented colonies

with apparently no contaminants present. To insure a pure culture two isolated colonies from the first serial streak were serially streaked onto a second petri plate containing veal infusion agar medium. This plate was incubated at 37°C and a visual examination of the plate after 24 hours showed abundant growth of the yellow pigmented colonies and no colonies of contaminating organisms.

A gram stain was performed on colonies of the second serial streak plate. Microscopic examination revealed the organism to be gram positive and occurring in irregular masses of cells. Since this conformed to the description given for the genus Micrococcus in Bergey's Manual of Determinative Bacteriology (7th Ed., 1957), it was concluded that the purchased culture was Micrococcus lysodeikticus.

The veal infusion broth was prepared by infusing one pound of finely ground lean veal in one liter of distilled water at 4°C for 24 hours. The mixture was strained through cheesecloth and the filtrate heated at 80°C for one hour. The resulting coagulated proteins were removed by straining through several layers of cheesecloth, and the filtrate was diluted to one liter with distilled water.

Yeast water was prepared by placing 75 grams of Fleishman's compressed baking yeast in one liter of distilled water and boiling for 30 minutes. The mixture was filtered through Whatman No. 1 filter paper, and after autoclaving the filtrate was stored in the refrigerator at 2 ± 2°C.

The veal infusion agar medium was prepared by placing into a graduated cylinder: 100 ml of the veal infusion broth, 100 ml of the yeast water, 5 g of NaCl, 5 g of peptone, 20 g of agar and diluting to

one liter. This mixture was heated until a fairly clear solution was obtained, and then 2 g of glucose were added. The agar medium was autoclaved for 15 minutes, cooled, and stored at 2 ± 2°C.

Agar slants were prepared in 25 x 195 mm pyrex test tubes from the autoclaved medium. Micrococcus lysodeikticus from the second serial streak plate was inoculated onto 12 slants, and after incubation at 37°C for 24 hours the inoculum in each tube was harvested in 3 ml of pH 6.2 Sorenson's phosphate buffer (Emmel et al, 1964).

The resulting suspension was allowed to flow down the rough surface of a piece of nonsterile obscure glass placed eight inches from an ultraviolet lamp with 85 to 90 percent of the wavelengths about 2,537 angstroms. This procedure was repeated a second time to insure that most of the cells were killed. The killed-cell suspension was shell-frozen (Smolelis and Hartsell, 1949) and lyophilized until dry. The tubes were fire-sealed while still under a vacuum and stored at 2 ± 2°C.

The lyophilized M. lysodeikticus cells were rehydrated just prior to use with pH 6.2 Sorenson's phosphate buffer and well mixed to obtain a uniform suspension. The turbidity of the suspension was adjusted with pH 6.2 phosphate buffer to read 1 optical density against a distilled water blank at a wavelength of 540 mu. A Bausch and Lomb Spectronic 20 was used to perform all spectrophotometric readings.

A standard curve for lysozyme (Figure 1) was determined so that the lysozyme concentration in the root exudate could be calculated. A 1:10,000 stock solution was prepared by dissolving 0.1 g of crystalline

Figure 1. Standard Lysozyme Curve Showing Lysozyme Concentration and the Dilution Factors Versus the Optical Density.

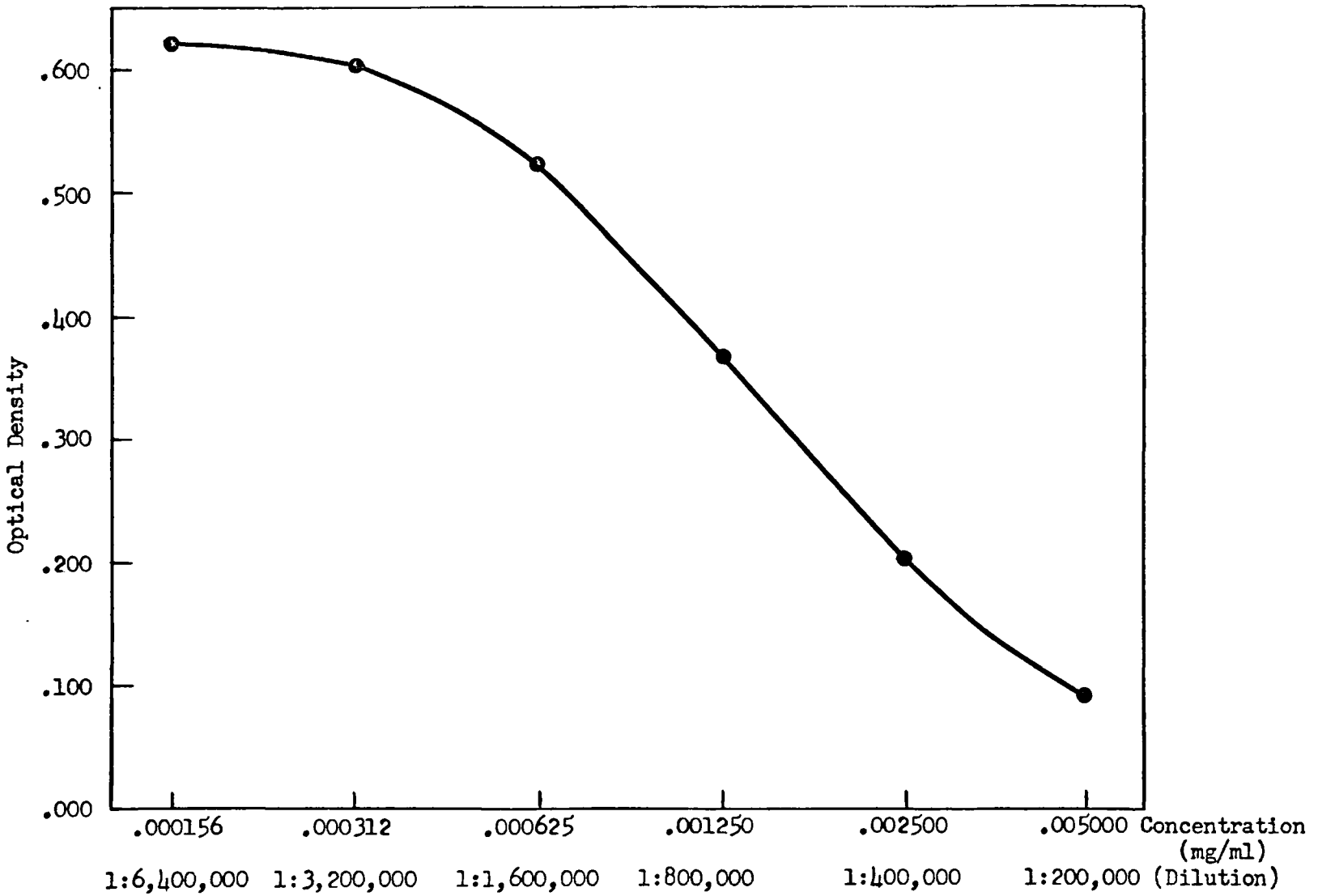


Figure 1. Standard Lysozyme Curve Showing Lysozyme Concentration and the Dilution Factors Versus the Optical Density.

lysozyme (Calbiochem lot number 54106) per liter of pH 6.2 Sorenson's phosphate buffer. From the stock solution twofold dilutions were made with pH 6.2 phosphate buffer starting with 1:200,000 and progressing to 1:6,400,000. This gave a range of 0.00500 mg to 0.000156 mg per ml of dilution. A 2 ml aliquot of each dilution was added to a 2 ml aliquot of the 1 optical density solution of M. lysodeikticus substrate. This mixture was inverted several times in a cuvette and the cuvette was placed in a 26°C water bath for 20 minutes before reading against a distilled water blank. All trials were duplicated in matched cuvettes.

The optical density of a control consisting of 2 ml of the pH 6.2 phosphate buffer and 2 ml of the substrate was determined for calculation purposes. This control will henceforth be referred to as the standard control.

The experimental plant was tomato, Lycopersicon esculentum L., variety Bonnie Best (stock number 9560, lot number 313927, George J. Ball, Inc., West Chicago, Illinois). The seeds were germinated in flats of vermiculite, and when they had attained a height of from 2.5 to 5 cm they were transferred to larger flats of vermiculite and allowed to grow until they had reached a height of 15 to 17.5 cm. The roots were washed free of vermiculite with distilled water, and the plants were placed into 2 quart jars containing a modified Hoaglands nutrient solution (Meyer et al, 1955). Iron was supplied as Fe-EDTA in a concentration of 5 ppm. Air was bubbled through the solutions from 7 A.M. to 6 P.M. daily. All of the growing operations were carried out in a greenhouse with the temperature maintained above 11°C in

the winter and around 29°C in the summer. The plant ages were determined from the day of planting.

When the plants had attained the ages indicated in the tables they were transferred to the laboratory. In the first experiment, three 70-day old root systems were placed in 800 ml of demineralized distilled water containing lysozyme at a concentration of 0.1 mg/ml. A control was set up in 800 ml of distilled water only.

In the second experiment, three 70-day old root systems were placed in modified Hoagland's solution containing lysozyme at a concentration of 0.1 mg/ml. A control was set up with a 70-day old root system in modified Hoagland's solution only. The stems of the plants were excised with a razor blade 3 cm above the uppermost root. A piece of 15 cm long tygon tubing with a diameter necessary to obtain a tight fit was inserted over the stem stump and a rubber band was tightly wrapped around the area. The tubing was held in place by means of a cork stopper fitted into the mouth of the vessel. Approximately 80 to 90 percent of the root area was covered by the lysozyme solution. The roots were left exposed to the lysozyme solution for 3 to 6 hours until at least 3.5 ml of exudate was collected. The exudate was removed from the tygon tubing with a Pasteur pipette, placed into a test tube, and analyzed immediately.

McLaren, Jensen, and Jacobson (1960) suggested that the pH of the surrounding solution might influence the adsorption of proteins onto the root surface and thus affect uptake. To examine this hypothesis a third experiment was designed similar to the two previous ones

except that 0.067M Sorenson's phosphate buffers of pH 5, 6, 7, 8, and 9 were substituted for the demineralized distilled water and the modified Hoaglands solution. No spectrophotometric analysis was performed, however, since no exudate was obtained.

A series of experiments were performed in the pressure chamber shown in Figure 2. This chamber consisted of a cylindrical vessel (A) surmounted by a flat circular lid (B). An air-tight seal was effected by a circular rubber O-ring (C) mounted in the lip of the vessel. A total of 5 brass nozzles (E) were screwed into the lip and sealed air-right with rubber O-rings (D). Compressed air was admitted through the air inlet valve and exhausted through the air exit valve.

The tomato plant stems were excised 3 cm above the uppermost root and a piece of tygon tubing 15 cm long (F) was inserted over the stem stump. The inner diameter of the tubing chosen was that needed to obtain a snug fit. The tygon tubing was further sealed by tightly wrapping and tying a rubber band around the stump area. A piece of rubber tubing (G) was fitted onto the brass nozzle and sealed with a wrapped and tied rubber band. The tygon tubing was pushed through both the rubber tubing and the brass nozzle. The rubber tubing was sealed against the tygon tubing by a wrapped and tied rubber band.

The lid holding the root systems was placed on the vessel so that the root systems were almost fully submerged in a phosphate buffer solution containing 0.6 mg/ml of lysozyme (McLaren et al, 1960). When 250 ml beakers were employed 125 ml of lysozyme solution were used, and when 125 ml beakers were employed 50 ml of lysozyme solution were used.

Figure 2. Diagram of Pressure Chamber (Not to Scale)

A) Cylindrical Vessel
B) Circular Lid
C) RubberO-ring Seal for Lid
D) Rubber O-ring Seal for Brass Nozzle
E) Brass Nozzle
F) Tygon Tubing
G) Rubber Tubing
H) Wooden Platform
J) Pressure Gauge
K) Wing Nut

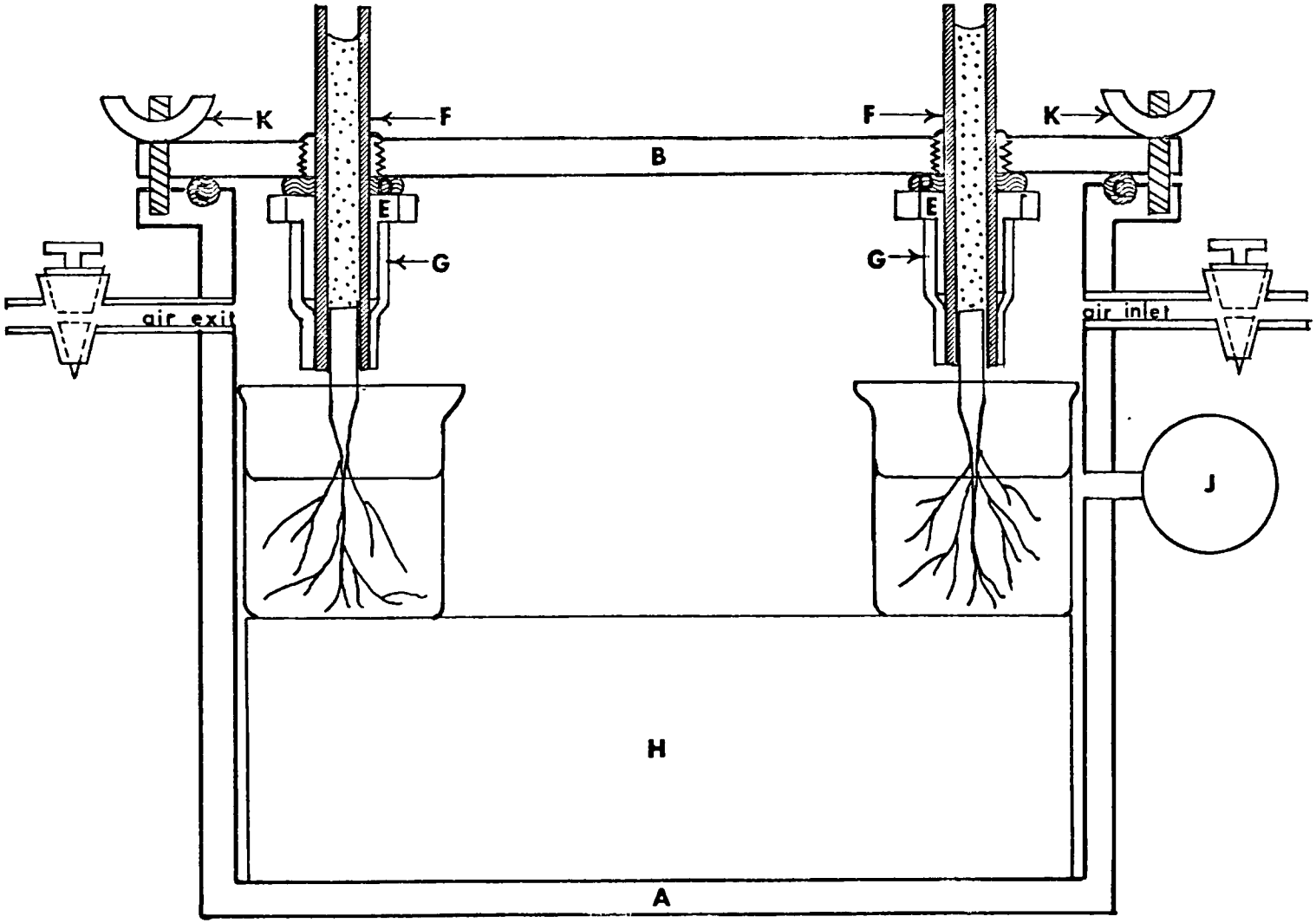


Figure 2. Diagram of Pressure Chamber

The height of the beakers was adjusted by means of a wooden platform (H) in the bottom of the vessel so that the tubing would not be submerged in the lysozyme solution. The lid was secured onto the vessel by means of 6 wing nuts (K), and air from a commercially supplied compressed air tank was admitted into the chamber until a pressure gauge (J) mounted on the side of the vessel indicated 2 atmospheres. The root systems were left at this pressure until the first 2.5 to 6 ml of exudate were obtained. The time necessary to obtain this amount of exudate ranged from 30 minutes to 8 hours.

The exudate was removed from the tygon tubing with a Pasteur pipette often enough to prevent the exudate from running over. The removed exudate was placed into a graduated test tube, the volume recorded, and the tube immediately frozen to preserve enzyme activity. Upon obtaining sufficient exudate the air was released from the chamber by means of the air exit valve. The root systems were removed from the tubing, and the root volumes were measured by means of water displacement in a 50 ml graduated cylinder. As much water as possible was removed from the surface of the roots by gently pulling the root system up between the thumb and index finger.

A series of experiments involving DMSO was conducted in the pressure chamber in the manner previously described. Root systems were placed in phosphate buffers containing DMSO and lysozyme. The DMSO was supplied at concentrations of 0.001M or 0.01M, and the lysozyme was supplied at 0.6 mg/ml.

The bioassay was performed by placing 2 ml of the rehydrated cell suspension and 2 ml of the root exudate into cuvettes. The

cuvettes were well mixed and placed into a 26°C water bath for 20 minutes. After this time the suspension was read on the spectrophotometer against a distilled water blank. Substrate-buffer controls, prepared in the same manner as those in the standard curve preparation, were run with each group of exudates assayed. These controls will, henceforth, be referred to as trial controls.

The percent transmittance values for the standard control, the trial controls, and the assayed exudate values were set up as a direct proportion and new exudate values calculated. The percent transmittance was converted back to optical density and these optical density values are shown in the tables. These standard control based calculations were performed in order to adjust for any variances in the turbidity of the substrate between the various trials. This would, in effect, make the exudate values more comparable.

RESULTS AND DISCUSSION

Analysis of the exudate from the first two experiments involving lysozyme in distilled water or modified Hoagland's solution indicated that no protein was being taken up. It was thought that perhaps enough mass water flow would be induced across the root to move mechanically the protein into the stele where it could be translocated to the cut surface of the stem by root pressure. However, from the results apparently this was not the case.

When detopped root systems were placed in 0.067M phosphate buffer at different pH's and air was bubbled through the solution during root exposure, the quantity of exudate obtained was not sufficient for analysis. The modified Hoagland's solution was calculated to be 0.009M which was one-seventh that of the phosphate buffer. It would appear that the presence of the phosphate buffer increased the osmotic potential of the ambient solution enough to virtually cease water uptake. Kozlowski (1964) has observed that small increases in the osmotic potential of the soil solution cause large decreases in water uptake. Bradfute and McLaren (1964) found that a concentration of 0.067M phosphate buffer permitted about 50 percent of the lysozyme supplied to be taken up by excised 5-day old barley roots. The use of 70-day old tomato root systems rather than 5-day old barley root tips could have caused the discrepancy.

Tables 1 through 3 show the results of the first three trials conducted under pressure chamber conditions on root systems about 10 to 11-weeks old. The average lysozyme concentration values and ± one standard deviation was computed for each pH and these values are illustrated in Figure 3. The standard deviations indicate no significant difference between pH's 5, 6, and 7. However, the uptake of lysozyme at pH's 8 and 9 was significantly higher than at pH's 5, 6, and 7. It should be noted that ± one standard deviation has a confidence level of 68 percent.

The ability of younger root systems to take up lysozyme was tested, and the results are shown in Tables 4 and 5. Table 4 indicates that 7 to 8-week old root systems do not take up lysozyme as readily as the older roots. At pH 9 the 7 to 8-week old roots showed 50 percent less uptake than the 10 to 11-week old roots. No lysozyme was detected in the exudate at pH 8 in the 7 to 8-week old plants. The root systems in pH's 5, 6, and 7 did not exhibit any uptake. Table 5 indicates that for 5-week old roots no uptake was obtained for pH 9. The uptake at pH 8 was about one-fourth as much as the average uptake at pH 8 in the 10 to 11-week old roots. There was more uptake at pH's 6 and 7 than at the same pH's in the older roots; however, these results may be invalid for reasons which will be discussed later.

Table 6 indicates that for 7-week old roots with 0.001M DMSO added, uptake at pH's 8 and 9 is similar to that exhibited in the 10 to 11-week old roots. When these results are compared to the uptake obtained for root systems of comparable age, it appears that DMSO has an enhancing effect (compare Tables 4 and 6).

TABLE 1

TOMATO PLANT ROOTS ABOUT 10 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE
(Trial 1)

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
68	5	0.140	.622	0.000156
69	6	0.000 *	.392	0.001040
68	7	0.067	.297	0.001670
70	8	0.060	.206	0.002380
69	9	0.189	.194	0.002500

*ml of exudate for this trial not recorded.

TABLE 2

TOMATO PLANT ROOTS ABOUT 11 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

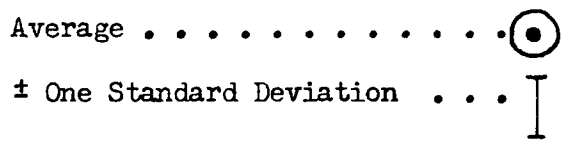
Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
79	5	0.120	.289	0.001610
80	6	0.155	.426	0.000833
81	7	0.304	.492	0.000695
82	8	0.181	.200	0.002500
83	9	0.290	.174	0.002700

TABLE 3

TOMATO PLANT ROOTS ABOUT 10 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE
(Trial 2)

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
71	5	0.081	.660	-0-
71	6	0.087	.660	-0-
70	7	0.095	.660	-0-
70	8	0.088	.211	0.002380
69	9	0.110	.180	0.002630

Figure 3. Lysozyme Concentration in Exudate From 10 to 11-week Old Tomato Roots. Each Point Represents the Average of Three Root Systems. A Pressure of 2 atm Was Applied to the Solution Around the Roots.



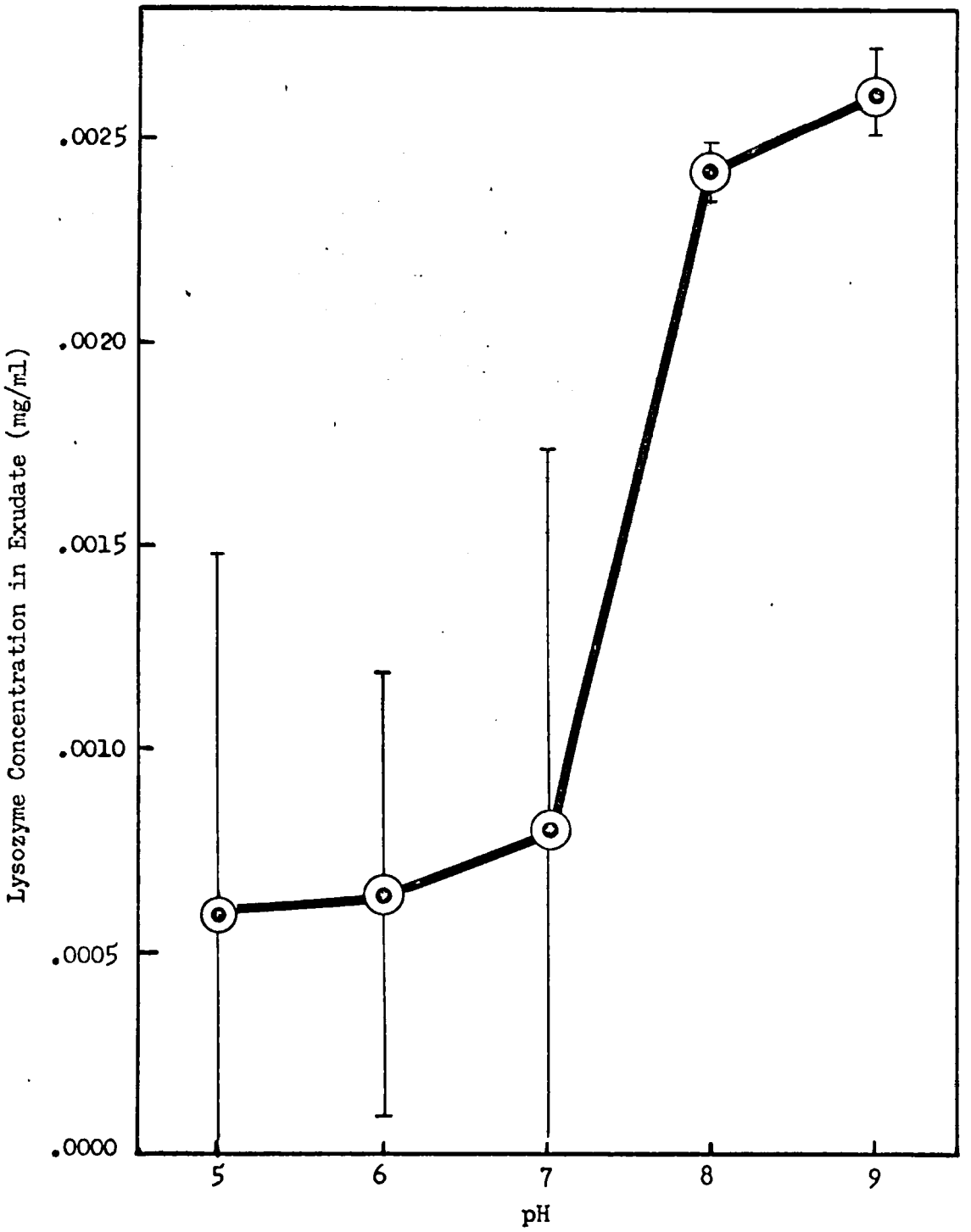


Figure 3. Lysozyme Concentration in Exudate From 10 to 11-Week Old Tomato Roots.

TABLE 4

TOMATO PLANT ROOTS ABOUT 7 TO 8 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
53	5	0.045	.660	-0-
54	6	0.044	.660	-0-
55	7	0.073	.660	-0-
56	8	0.044	.660	-0-
56	9	0.062	.460	0.00104

TABLE 5

TOMATO PLANT ROOTS ABOUT 5 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
30	5	0.058	.660	-0-
31	6	0.067	.399	0.001000
33	7	0.093	.253	0.001920
38	8	0.036	.529	0.000595
38	9	0.029	.660	-0-

TABLE 6

TOMATO PLANT ROOTS ABOUT 7 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME AND 0.001M DMSO IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
51	5	0.332	.660	-0-
51	6	0.201	.656	-0-
51	7	0.284	.660	-0-
51	8	0.316	.168	0.00278
51	9	0.226	.200	0.00250

The conditions under which the data in Table 7 were obtained were the same as those under which the data in Table 6 were obtained except that the DMSO concentration was raised to 0.01M. At this concentration there does not seem to be any remarkable enhancement over the uptake at a concentration of 0.001M. When 5-week old roots were placed in 0.01M DMSO solutions, the uptake at pH 9 was about half as much as the uptake of 7-week old roots in 0.01M DMSO at pH 9. The uptake at pH 8 was essentially the same. These data are presented in Table 8.

The results shown in Table 9 are those of 3-week old roots in 0.01M DMSO. Here, again, a little uptake is noted at pH's 8 and 9, and greater uptake is evident at pH's 5 and 7.

The values for pH 5 in Table 9 and those for pH's 6 and 7 in Table 5 are large enough to suspect that perhaps a root or roots could have been broken during some phase of the experiment. A break in the system could allow the lysozyme solution to be forced directly into the xylem tissue and thus higher than usual concentrations of lysozyme would appear in the exudate. Every precaution was taken to insure against root breakage, but separations could have occurred which were not detected.

It appears from the results that higher uptakes occur in older root systems, at higher pH's and at lower DMSO concentrations. It is known that when secondary growth and branching occurs in roots of herbaceous dicots separation occurs in the endodermis and the cortical tissue (Hayward, 1938). Under conditions of pressure protein molecules could gain entry into the xylem by passing through expanded intercellular spaces of the cortex and crushed cells of the endodermis.

TABLE 7

TOMATO PLANT ROOTS ABOUT 7 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME AND 0.01M DMSO IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
53	5	0.126	.660	-0-
53	6	0.140	.660	-0-
53	7	0.119	.520	0.000625
53	8	0.134	.354	0.001280
53	9	0.353	.190	0.002560

TABLE 8

TOMATO PLANT ROOTS ABOUT 5 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME AND 0.01M DMSO IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age in Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
38	5	0.071	.660	-0-
38	6	0.066	.660	-0-
39	7	0.079	.600	0.00036
39	8	0.066	.219	0.00119
40	9	0.200	.204	0.00119

TABLE 9

TOMATO PLANT ROOTS ABOUT 3 WEEKS OLD SUPPLIED WITH 0.6 mg/ml LYSOZYME AND 0.01M DMSO IN PHOSPHATE BUFFER AND PLACED UNDER 2 atm PRESSURE

Plant Age In Days	pH of Lysozyme Solution	ml Exudate/Cm^3 Root/Hour	Optical Density of Substrate Suspension	Lysozyme Concentration (mg/ml) in Root Exudate
18	5	0.088	.110	0.004500
18	6	0.061	.630	-0-
20	7	0.061	.432	0.001030
20	8	0.900	.500	0.000719
23	9	0.720	.619	0.000156

It has been speculated by Collander that pH does have a modifying effect on cell membranes,

> . . . the uptake of weak acids and bases is strongly influenced by the pH of the ambient solution. This, however, is primarily an influence on the ionization of the penetrating substances rather than an influence on the cells themselves. On the other hand, if the proteins of the plasmalemma participate at all in the uptake of ions, the pH of the medium may be expected to affect cell permeability, since it is well known that proteins on the acid side of their isoelectric point will combine with anions, while on the alkaline side cations are adsorbed. Experimental evidence of such effects is, however, meager. . .

and this may be a reason for the increased uptake at the higher pH values.

McLaren (1960) indicated that plant roots are thought to have negative surface charges, and a protein below its isoelectric point, being positively charged, would be more readily adsorbed onto the root surface. One would suspect then that the lower pH's would have the most adsorption and, therefore, the greatest uptake. However, the larger concentration of hydrogen ions at the lower pH's probably compete with the protein for the negative sites on the root surface. Therefore, proteins in buffer solutions at pH's just below the isoelectric point may have a better chance to be adsorbed than those in solutions at lower pH's. This may be the case with lysozyme which has an isoelectric point of about 10.5.

Not much is known about the effects of DMSO on cell membranes other than that it seems to render them more permeable to certain molecules. The results obtained here indicate that at certain pH's DMSO seems to facilitate the passage of lysozyme molecules across young root tissue. Whether this movement of lysozyme actually involves

penetrating a membrane somewhere in the pathway is not revealed by the results obtained here.

Since in most instances only one experiment was conducted under a given condition the results should be regarded only as possible trends. Further experimentation with increased replications might yield results that would show better statistical confidence than is presented here.

SUMMARY

1. Tomato roots were exposed to a 0.1 mg/ml solution of lysozyme in demineralized water, modified Hoagland's solution, or 0.67M Sorenson's phosphate buffers at pH's 5, 6, 7, 8, and 9. The results indicated that no lysozyme was taken up.

2. Tomato roots were supplied with a 0.6 mg/ml lysozyme solution in Sorenson's phosphate buffer at pH's 5, 6, 7, 8, and 9, placed in a pressure chamber and subjected to 2 atm of air pressure. The results indicated that the best uptake occurred in roots about 10 to 11 weeks old as compared to roots 5 and 7 weeks old and at pH's of 8 and 9 as compared to 5, 6, and 7.

3. Dimethyl sulfoxide was added to the lysozyme-phosphate buffer solution and 2 atm of pressure applied. The results indicated that the best uptake occurred in younger roots and at lower DMSO concentrations.

4. It was speculated that possible secondary root growth in the older roots may have crushed some of the endodermis and expanded some of the intercellular spaces, thus admitting to the xylem the lysozyme under pressure.

5. The higher pH's may have allowed lysozyme to be adsorbed onto the root surface or may have caused membrane modification, thus allowing more lysozyme through the cells.

6. Dimethyl sulfoxide may have caused membrane modification or may have carried the lysozyme into the xylem in a "piggy-back" fashion.

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