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B. T. GALLOWAY, *Chief of Bureau.*

ABSORPTION AND EXCRETION OF SALTS BY ROOTS, AS INFLUENCED BY CONCENTRATION AND COMPOSITION OF CULTURE SOLUTIONS.

I.—CONCENTRATION RELATIONS OF DILUTE SOLUTIONS OF CALCIUM AND MAGNESIUM NITRATES TO PEA ROOTS.

BY

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DRUG-PLANT, POISONOUS-PLANT, PHYSIOLOGICAL, AND FERMENTATION INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., July 24, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 231 of the special series of this Bureau a manuscript by Dr. Rodney H. True, Physiologist, and Mr. Harley Harris Bartlett, Chemical Biologist, entitled "Absorption and Excretion of Salts by Roots, as Influenced by Concentration and Composition of Culture Solutions. I.—Concentration Relations of Dilute Solutions of Calcium and Magnesium Nitrates to Pea Roots."

This paper, submitted by the Physiologist in Charge of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, is to be followed by others under the same general title. The entire series will deal with problems of plant nutrition which have an important bearing upon the theory and practice of agriculture.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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ABSORPTION AND EXCRETION OF SALTS BY ROOTS, AS INFLUENCED BY CONCENTRATION AND COMPOSITION OF CULTURE SOLUTIONS.

I.—CONCENTRATION RELATIONS OF DILUTE SOLUTIONS OF CALCIUM AND MAGNESIUM NITRATES TO PEA ROOTS.

INTRODUCTION.

In the course of work carried on several years ago by R. H. True in collaboration with Lyman J. Briggs, the change in salt concentration of culture solutions in which seedlings of *Lupinus albus* were grown was followed during the growth of the plants by means of the method of electrical conductivity. The results (which have never been published) indicate that lupine roots, when grown in distilled water, excrete electrolytes which render the water so used a better medium than fresh distilled water for the growth of a second set of seedlings. This clue has led to the use of the conductivity method in studying the influence of concentration on absorption and excretion of salts by roots of plants growing in dilute culture solutions. This bulletin reports the results of a series of experiments with field peas grown in solutions of calcium and of magnesium nitrates. It is to be followed by studies of other plants and of other solutions. Future work will be directed toward determining the chemical composition of the culture solutions after plants have grown in them, and also of the plant roots themselves, in the hope that the results may throw light upon the mechanism of permeability. The purely physical results already obtained, however, are of so much interest that they are presented at this time.

CULTURE METHODS.

On account of the great dilution of the solutions used (varying from $\frac{M}{5,000}$ to $\frac{M}{500,000}$) it was necessary to make several modifications of the usual water-culture technique. All cultures were made in liter beakers of Jena glass, boiled out with distilled water. In each beaker were placed 520 c. c. of distilled water. Two paraffined glass rods, crossing each other in the form of a letter X, of such length that their ends projected about 2 cm. from the water, served as supports for a cake of paraffin. This cake was made by pouring enough

melted paraffin (melting point 65°) on the surface of the water to make a layer 7 or 8 mm. thick. After this layer had cooled and had drawn away from the sides of the beaker it was covered by a second layer of soft paraffin (melting point 45°) as thick as the first. The

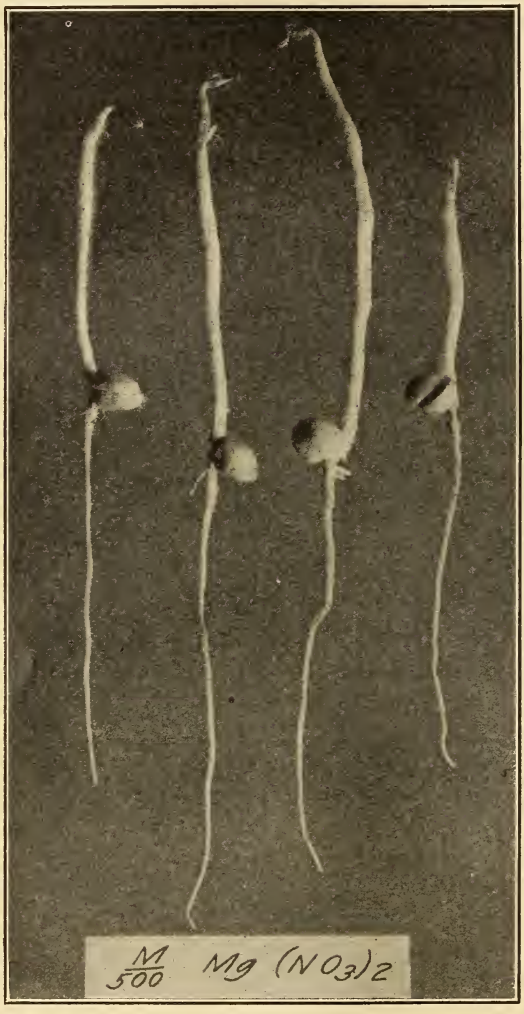

FIG. 1.—Seedlings of culture 4, experiment C.

hard paraffin afforded the necessary rigidity; the soft, which adhered to the glass, served to hold the cake firmly in place. Holes about 5 mm. in diameter were bored through the paraffin support. Four holes were equally spaced from the sides of the beaker and a fifth one was bored against the glass to serve as a pour-out.

A series of beakers with paraffin partitions was made ready for an experiment by rinsing the beakers with water of known resistance, which was poured from one beaker into the next until it had passed through all. This process was repeated until the resistance of the waste water when poured from the last beaker was no lower than at the start.

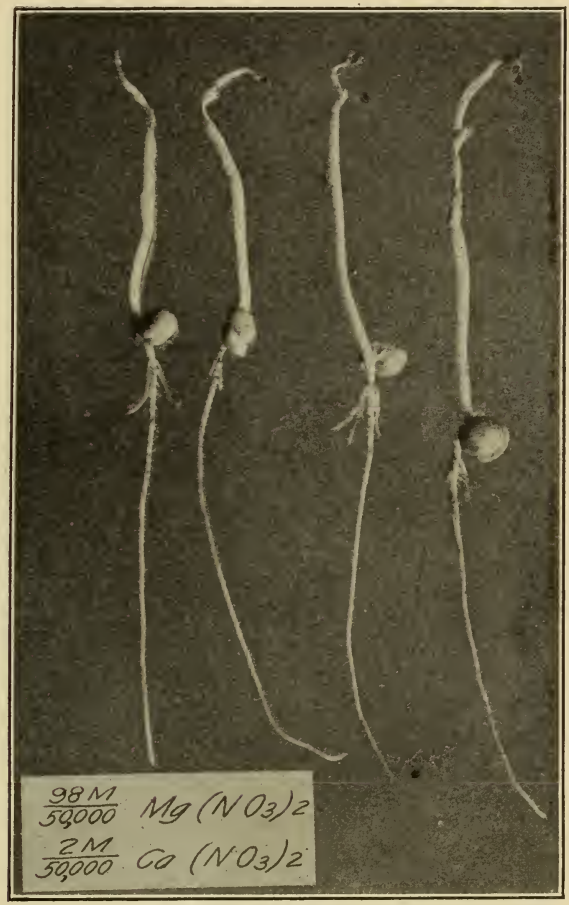

FIG. 2.—Seedlings of culture 6, experiment C.

The solutions for each series of cultures were always made from the same supply of distilled water, by diluting stock solutions of tenth molecular concentration. For measuring, flasks of Jena glass were used; for storing water, use was made of glass bottles which had been long in service and were very insoluble. At the outset of the work it was found that peas grew well in water from an automatic laboratory still which discharged into a glass container. Comparative experi-

ments showed that this water was measurably improved as a culture medium by a double redistillation according to the method of Richards,[1] i. e., by using water-seal connections between Jena glass boilers and block-tin condensers. It was distilled first from alkaline and then from acid potassium permanganate. In comparing the effect of the two kinds of water, the rate of elongation of the primary root was

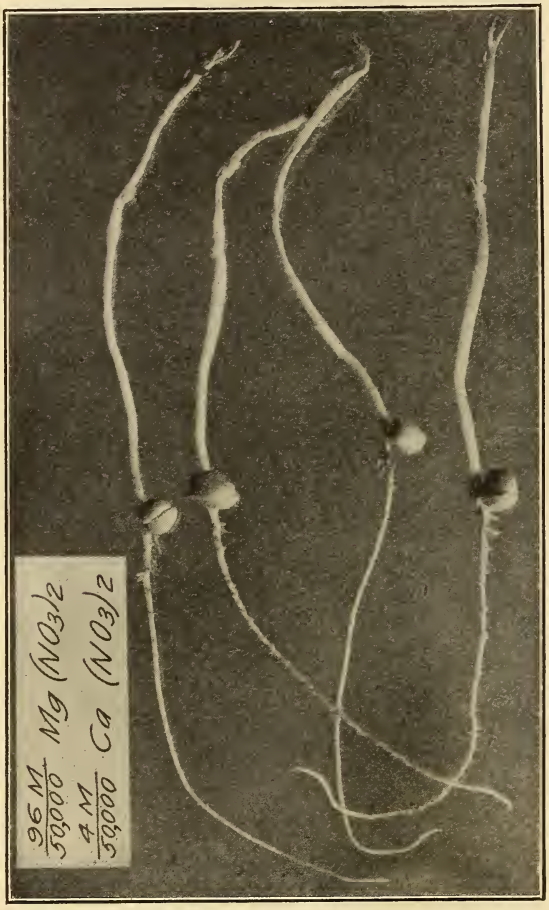

FIG. 3.—Seedlings of culture 8, experiment C.

made the criterion. The average elongation for 24 peas during the first 24 hours was 12.6 mm. in thrice-distilled water, and 9.2 mm. in the laboratory water. The average elongation for 24 white lupines in thrice-distilled water during the first 24 hours was 18 mm., and during the second 24 hours 4 mm.—a total growth of 22 mm. in 48

[1] Richards, T. W. Proceedings of the American Academy of Arts and Sciences, vol. 30, 1894, p. 380.

hours. In laboratory water the growth was 16.9 mm. for the first 24 hours and 5 mm. for the second 24 hours—a total growth of 21.9 mm. Pea cultures a week old were to outward appearance as good in one kind of water as the other, and the experiments were therefore carried on with the laboratory water, without any preliminary treatment, such as shaking it with carbon black.

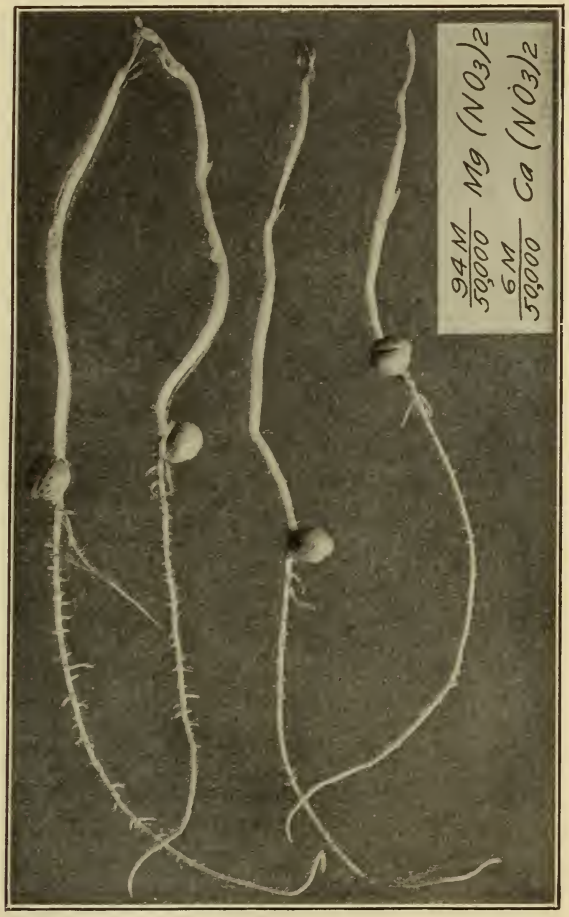

FIG. 4.—Seedlings of culture 10, experiment C.

Canada field peas, supplied by Prof. C. V. Piper (S. P. I. No. 19290), proved to be very easily handled in water cultures. Before germination they were graded according to size and color (some had green, others yellow, cotyledons). A sufficiently large number of the same grade were germinated in moist sphagnum, so as to have enough seedlings for a second selection at the time of setting up the cultures. They were used when the primary root had attained a length of 4 or

5 centimeters, but before any laterals had appeared. By very carefully removing the seed coats, it was found that sterile cultures could always be obtained, for the fungi and bacteria which sometimes attack the cotyledons generally appear first as saprophytes on the dead tissue of the seed coats, from which they infect the cotyledons. Four seedlings were placed in each beaker, after washing them twice with distilled water. The culture solutions were all made up to a volume

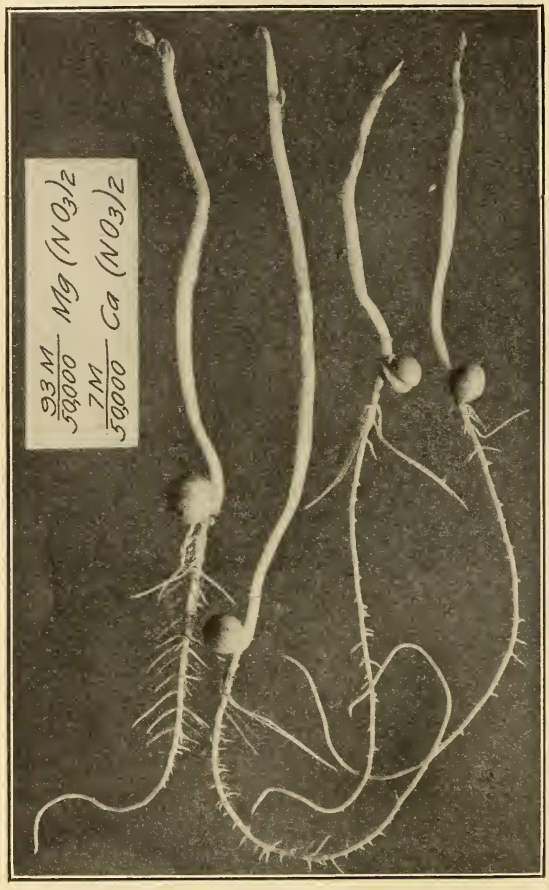

FIG. 5.—Seedlings of culture 11, experiment C.

of 500 c. c., so that an air space of 20 c. c. was left between the solution and the paraffin cake. When conductivity readings were made during the course of an experiment the culture solution was poured out through the hole at the side of the paraffin partition, and made up to 500 c. c. in order to replace the water lost by transpiration and evaporation. After determining its conductivity it was replaced without in the least disturbing the seedlings. The cultures were all kept in a dark chamber at laboratory temperature.

PLAN OF THE EXPERIMENTS.

Three preliminary experiments were carried through before making any conductivity measurements, in order to become acquainted with the specific reactions of pea roots to solutions of magnesium and calcium nitrates. In each of the first two experiments a series of 14 solutions was used.

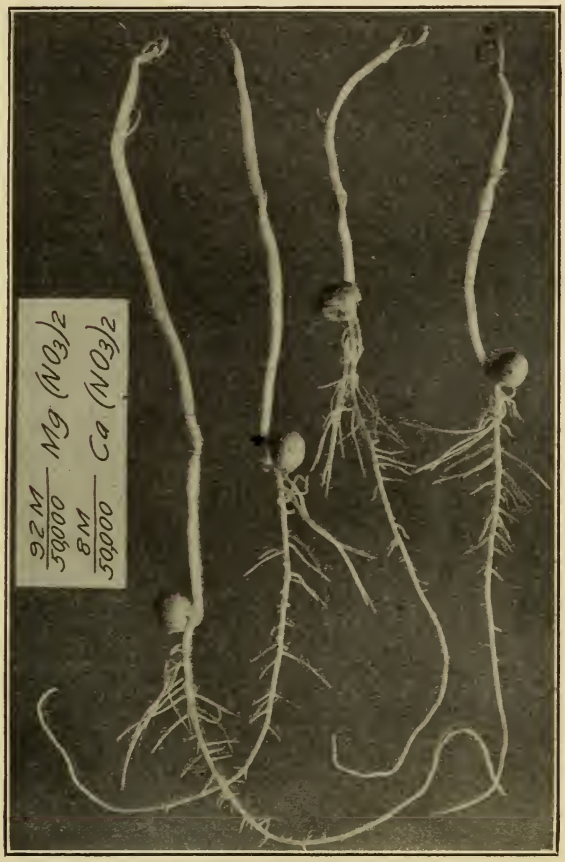

FIG. 6.—Seedlings of culture 12, experiment C.

EXPERIMENT A.

No. 1 was $\frac{M}{5,000}$ magnesium nitrate. Nos. 2 to 12 were all of equivalent concentration at $\frac{M}{500}$. No. 2 was pure magnesium nitrate, No. 3 was nine-tenths magnesium nitrate and one-tenth calcium nitrate, No. 4 eight-tenths magnesium nitrate and two-tenths calcium nitrate, etc. Thus each of the 11 cultures of equivalent concentration dif-

fered from the one immediately preceding, in that its concentration with regard to magnesium nitrate was less by $\frac{M}{5,000}$ and its concentration with regard to calcium nitrate greater by $\frac{M}{5,000}$. No. 12, of course, was $\frac{M}{500}$ calcium nitrate. No. 13, bearing the same relation-

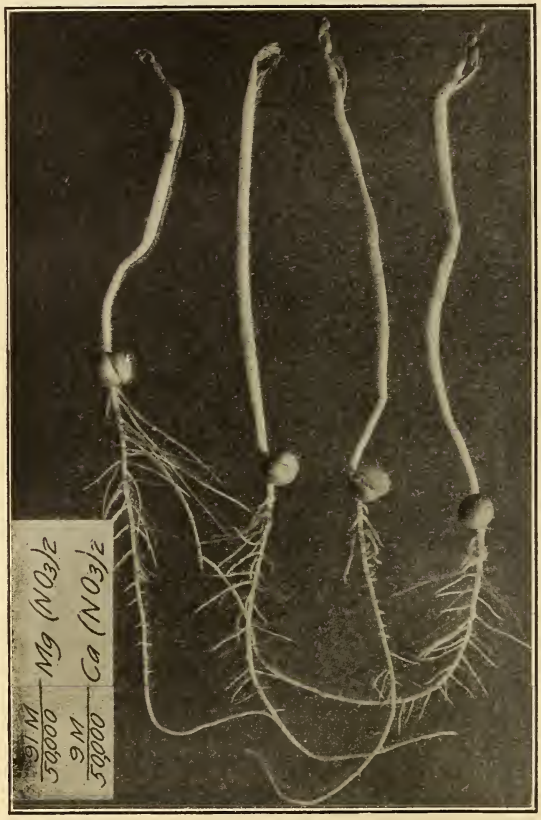

FIG. 7.—Seedlings of culture 13, experiment C.

ship to No. 12 as No. 1 to No. 2, was $\frac{M}{5,000}$ calcium nitrate. The two $\frac{M}{5,000}$ solutions of the pure salts (Nos. 1 and 13) were used in order to determine the effect, when used alone, of the smallest concentration of magnesium or calcium nitrate contained in any of the mixed solutions. No. 14 was distilled water. The cultures were carried seven days in experiment A. At the end of this period the condition of the plants was as follows:

No. 1 ($\frac{M}{5,000}$ $Mg(NO_3)_2$). Primary roots alive; laterals none.

No. 2 ($\frac{M}{500}$ $Mg(NO_3)_2$). Primary roots dead; solution discolored.

Nos. 3 ($\frac{9M}{5,000}Mg(NO_3)_2+\frac{M}{5,000}Ca(NO_3)_2$) to 12 ($\frac{M}{5,000}Mg(NO_3)_2+\frac{9M}{5,000}$ $Ca(NO_3)_2$). Root system finely developed; laterals long and flexuous.

No. 13 ($\frac{M}{5,000}Ca(NO_3)_2$). Roots as in Nos. 2 to 12.

No. 14 (distilled water). Root system well developed; laterals rigid, divaricate, and thicker than in Nos. 3 to 13.

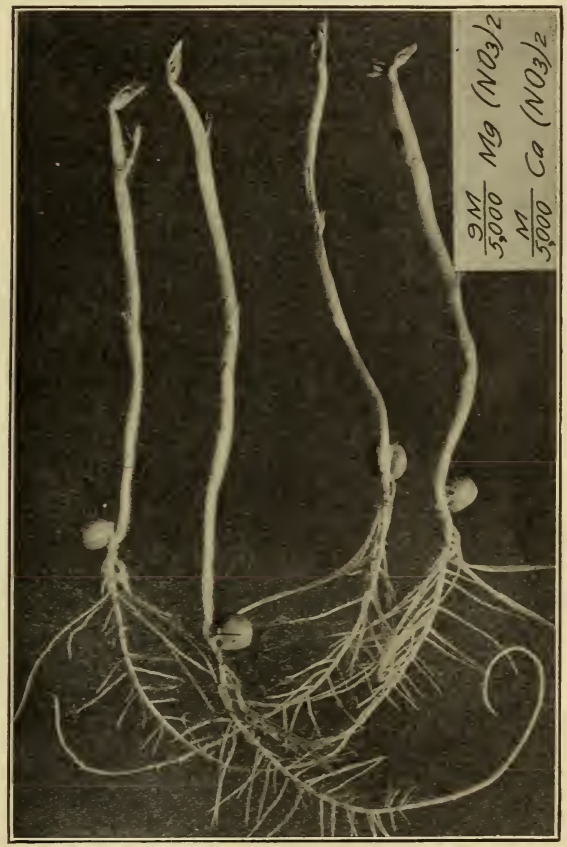

FIG. 8.—Seedlings of culture 14, experiment C.

The solutions of this series were made up with triple-distilled water (water from the laboratory still redistilled from alkaline and then from acid $KMnO_4$). Significant points to be noted are (1) that $\frac{M}{500}$ $Mg(NO_3)_2$ is extremely toxic, and (2) that the substitution of calcium for one-tenth of the magnesium in an $\frac{M}{500}$ solution makes it as good a

culture medium as $\frac{M}{500}$ $Ca(NO_3)_2$ alone. These facts are illustrated in Plate I, which shows one typical seedling from each of the 14 cultures, photographed at the end of the experiment.

EXPERIMENT B.

The series of cultures used in experiment B was identical with that of experiment A, except that the solutions were made up with water from the laboratory still. At the end of a week the results were the same as those obtained in experiment A. At the end of a month the cultures were in the following condition:

No. 1 ($\frac{M}{5,000}Mg(NO_3)_2$). Roots dead; stem wilted; three (or only two) pairs of branches had developed successively in the axils of the cotyledons, of which all but the youngest pair were wilted.

No. 2 ($\frac{M}{500}Mg(NO_3)_2$). Root and stem dead; cotyledons alive, without axillary branches.

No. 3 ($\frac{9M}{5,000}$ Mg+$\frac{M}{5,000}$ Ca). Roots well developed, living, but slightly discolored; stem wilted above; one pair of axillary cotyledonary branches.

Nos. 4 to 13. Roots like No. 3, but white; stem wilted above; no cotyledonary branches.

No. 14 (distilled water). Roots dead; plants much like those in No. 1, except for the presence of lateral roots.

EXPERIMENT C.

The first three cultures of the series used in experiment C were designed to determine the concentration at which magnesium nitrate alone exhibited an effect markedly more injurious than that of distilled water. No. 1 was distilled water; No. 2, $\frac{M}{50,000}$ Mg $(NO_3)_2$; No. 3, $\frac{M}{10,000}$ Mg $(NO_3)_2$. Cultures 4 to 14, designed to determine the highest ratio of magnesium to calcium which would permit lateral roots to develop perfectly, were of equivalent concentration at $\frac{M}{500}$, grading by tenths between cultures 2 and 3 of experiments A and B. The calcium concentration of each solution was greater by $\frac{M}{50,000}$ than that of the preceding solution, and the magnesium concentration correspondingly less. At the end of a week the plants had reached their maximum development. Nos. 4 and 14 (the same as Nos. 2 and 3 of experiments A and B) showed, as the corresponding cultures in the previous experiments had shown, the striking contrast between a dead unbranched primary root and

a luxuriantly developed root system with vigorous laterals. The intermediate cultures showed a perfect transition between these two conditions. The significant cultures from experiment C are shown in figures 1 to 8. The condition of the roots when photographed was as follows:

No. 4 ($\frac{M}{500}Mg(NO_3)_2$). Primary dead: no laterals.[1] (Fig. 1.)

No. 5 ($\frac{99\ M}{50,000}Mg+\frac{M}{50,000}Ca$). Hardly different from No. 4 in appearance, but alive.

No. 6 ($\frac{98\ M}{50,000}Mg+\frac{2\ M}{50,000}Ca$). Primordia of laterals beginning to show, but otherwise like No. 5. (Fig. 2.)

No. 7 ($\frac{97\ M}{50,000}Mg+\frac{3\ M}{50,000}Ca$). Similar to No. 6.

No. 8 ($\frac{96\ M}{50,000}Mg+\frac{4\ M}{50,000}Ca$). Laterals manifest. (Fig. 3.)

No. 9 ($\frac{95\ M}{50,000}Mg+\frac{5\ M}{50,000}Ca$). Laterals several millimeters long; condition of root system very similar to that of No. 3 ($\frac{M}{10,000}Mg\ (NO_3)_2$).

No. 10 ($\frac{94\ M}{50,000}Mg+\frac{6\ M}{50,000}Ca$). Laterals more numerous and somewhat longer than in No. 9. (Fig. 4.)

No. 11 ($\frac{93\ M}{50,000}Mg+\frac{7\ M}{50,000}Ca$). Three seedlings as in No. 10; one as in No. 12. (Fig. 5.)

No. 12 ($\frac{92\ M}{50,000}Mg+\frac{8\ M}{50,000}Ca$). With this ratio of magnesium to calcium the root system begins to present a normal appearance. The laterals in distilled water (culture No. 1) and in $\frac{M}{50,000}Mg\ (NO_3)_2$ (No. 2) reach a state of development intermediate between that attained in Nos. 11 and 12. (Fig. 6.)

No. 13 ($\frac{91\ M}{50,000}Mg+\frac{9\ M}{50,000}Ca$). Similar to the last. (Fig. 7.)

No. 14 ($\frac{90\ M}{50,000}Mg+\frac{10\ M}{50,000}Ca$). Root system perfectly developed. (Fig. 8.)

Conclusions which can be drawn from experiment C are: (1) That the maximum ratio of magnesium to calcium which permits a normal root development in $\frac{M}{500}$ solutions of the two nitrates alone is about $\frac{9}{1}$; (2) that magnesium nitrate in $\frac{M}{50,000}$ concentration has about the same effect on the roots as distilled water; (3) that $\frac{M}{10,000}Mg\ (NO_3)_2$ is approximately the most concentrated solution of this salt which permits the development of laterals.

[1] The short laterals shown just below the cotyledons in figures 1 to 8 were not developed in the culture solution, but in the moist air of the bore through the paraffin supports. They may be distinguished by their contorted growth from the long divergent laterals grown in the solutions.

Deduction as to plans.—Experiments A, B, and C showed that further work should be so planned as to study absorption and excretion (1) in solutions of magnesium nitrate more dilute than $\frac{M}{10,000}$; (2) in comparable solutions of calcium nitrate; (3) in equivalent solutions grading by tenths from $\frac{10}{10}$ Mg to $\frac{10}{10}$ Ca; (4) in equivalent solutions grading by hundredths from $\frac{100}{100}$ Mg to $\frac{90}{100}$ Mg $+\frac{10}{100}$ Ca; and (5) in solutions containing magnesium and calcium in constant ratio, but varying in concentration so as to be comparable with the solutions of magnesium alone and calcium alone.

RECORD OF THE EXPERIMENTS.

In the experiments recorded below the conductivity of the culture solutions was determined with the simplest form of the Wheatstone bridge, a scale 100 mm. in length with sliding contact. The apparatus specified by Ostwald and Luther[1] gave thoroughly satisfactory results. Sharp tone minima were obtained with solutions varying in resistance from 5,000 to more than 100,000 ohms. The familiar Arrhenius resistance cell was used, with bright platinum electrodes. In making a series of readings the known resistance was kept constant at 10,000 ohms. Because of the great range in concentration of the solutions, the tone minimum was frequently too far from the center of the bridge to be consistent with the highest accuracy. Nevertheless, the concentration changes in the culture solutions were comparatively of such magnitude that the error thus introduced and the error introduced by disregarding the small change in temperature during the course of an experiment could be disregarded. It is believed that the correction of small errors can have no significance in culture experiments involving the use of very dilute solutions, because the single great error due to the presence in the solutions of unknown or uncontrolled substances is greater than all other errors combined. Every culture solution contains, in addition to the known quantity of known substances, a variable quantity of other substances, dissolved from the apparatus or absorbed from the air. When solutions are dealt with which are as dilute as those used in our experiments (between $\frac{M}{250,000}$ and $\frac{M}{10,000}$ in concentration) the amounts of known and of unknown electrolytes are of the same order of magnitude. Consequently, when conductivity measurements show that minute amounts of material have been removed from a dilute solu-

[1] Ostwald, W., and Luther, R. Physiko-Chemische Messungen, 2d ed., p. 395 et seq.

tion, we have no means of knowing how much has been taken from the known components and how much from the unknown. Suffice it to say that unknown electrolytes are drawn upon to a considerable extent, for certain plants (e. g., beets) actually increase the resistance of distilled water in which they are grown.

The chief electrolytic impurity introduced into culture solutions from the air is carbonic acid. It is well known that water which is free from dissolved carbon dioxid can not be prepared by distillation in the air. Walker and Cormack[1] have shown that water in equilibrium with normal air has a total concentration $\frac{M}{80{,}000}$ with regard to actual and potential carbonic acid (H_2CO_3 and CO_2). Of this total, 14.4 per cent is dissociated into the ions $H+$ and HCO_3-, rendering the conductivity of the solution comparable with that of a $\frac{M}{500{,}000}$ solution of a univalent salt. In a water culture solution the partial conductivity due to dissociated carbonic acid would remain constant during an experiment except for the fact that roots are continually excreting carbon dioxid into the solution. With very dilute solutions there must be a considerable lag in the readjustment of the equilibrium between dissolved and atmospheric carbon dioxid. It is therefore probable that no other error in the work recorded in this paper is comparable in magnitude with that due to fluctuation in the carbon dioxid concentration of the solutions. It does not follow, however, that this error seriously vitiates the results; it is discussed merely in order to show that work with exceedingly dilute solutions can not be done with sufficient accuracy to warrant the correction of small experimental errors.

Since the culture solutions were not analyzed after our experiments, the conductivity readings afford only a rough idea of their salt content. In the statement of results the phrase "Concentration after growth" must be understood to mean that degree of concentration of the same salt or salts present in the solution before the growth of the plants which would give a solution having the same conductivity as that found to exist at the end of the experiment. Since, of course, the same salts are not present at the end of an experiment as at the beginning, the results as thus stated are at best only approximate. It is probable, for example, that in all the experiments reported below the salts excreted by the roots were in part univalent, whereas bivalent salts were absorbed. If a substitution of univalent salts for bivalent should take place equivalent for equivalent, the conductivity of a culture solution during growth might remain about the

[1] Walker, James, and Cormack, William. The Dissociation Constants of Very Weak Acids. Journal of the Chemical Society (London), vol. 77, pt. 1 (Transactions), 1900, pp. 5–21.

same, although the gram-molecular concentration would have become twice as great and the osmotic pressure higher by one-third.

In carrying out an experiment the conductivity of each solution was determined before use. The conductivities obtained at the end of the experiment were translated into terms of concentration of the original salt by interpolation and extrapolation into the original conductivity series; thus the error due to carbonic acid and unknown electrolytic impurities of the distilled water was eliminated as far as possible.

EXPERIMENT 1.

Experiment 1 embraced solutions grading by increments of $\frac{2\ M}{500{,}000}$ from distilled water to $\frac{26\ M}{500{,}000}$ Mg $(NO_3)_2$; duration of cultures, 9 days; temperature of solutions 19.5° at beginning of experiment, 21° at end.

TABLE I.—*Statement of results, experiment 1.*

Number of culture	1	2	3	4	5	6	7
Concentration:							
Before growth	0	$\frac{2\ M}{500,000}$	$\frac{4\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{12\ M}{500,000}$
After growth	$\frac{22\ M}{500,000}$	$\frac{13\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{11\ M}{500,000}$
Number of culture	8	9	10	11	12	13	14
Concentration:							
Before growth	$\frac{14\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{22\ M}{500,000}$	$\frac{24\ M}{500,000}$	$\frac{26\ M}{500,000}$
After growth	$\frac{8\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{11\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{22\ M}{500,000}$

These results show that pea roots excrete more electrolytes than they absorb when grown in solutions of magnesium nitrate weaker than $\frac{12\ M}{500{,}000}$; and, conversely, that they absorb more than they excrete when grown in solutions more concentrated than $\frac{12\ M}{500{,}000}$. This showing is made more evident to the eye by plotting the results. (Fig. 9.) The point at which the curve of change in concentration intersects the horizontal axis indicates the concentration (about $\frac{12\ M}{500{,}000}$) of magnesium nitrate at which the roots excrete and absorb electrolytes at approximately the same rate.

EXPERIMENT 2.

Experiment 2 embraced a series of $Ca(NO_3)_2$ solutions, grading from distilled water to $\frac{24\ M}{500,000}$. Conductivities were read at the end of 7 days and again at the end of 14 days from the date of starting. Temperatures: 22.5° at start, 23.2° after 7 days, and 23.4° after 14 days.

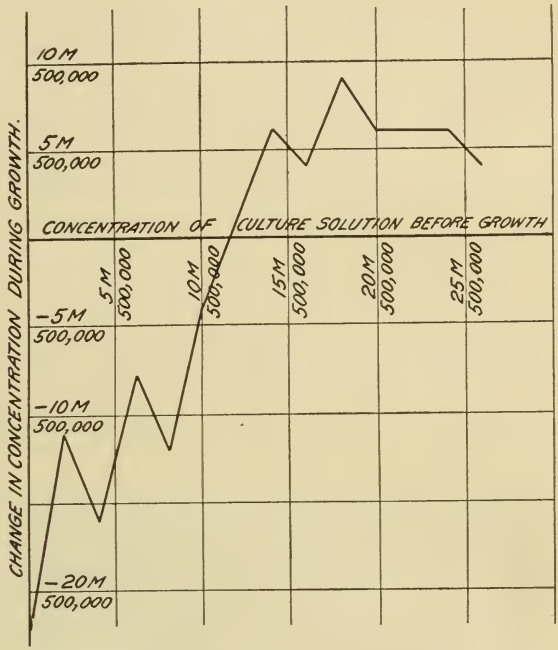

FIG. 9.—Curve showing relation of initial concentration of culture solutions to absorption and excretion of salts during growth in solutions of $Mg(NO_3)_2$ grading by increments of $\frac{2\ M}{500,000}$ from distilled water to $\frac{26\ M}{500,000}$.

This series indicates that the concentration at which income and outgo balance one another is rather less for calcium nitrate than for magnesium nitrate, being $\frac{7\ M}{500,000}$ as compared with $\frac{12\ M}{500,000}$. Moreover, it shows that the roots have a much higher efficiency in absorbing from calcium than from magnesium solutions when the concentration of the original solution is greater than the equilibrium concentration. This is what would be expected from a consideration of the fact that all the magnesium solutions used in experiment 1 had a distinctly toxic effect as compared with distilled water, whereas the effect of the calcium was decidedly stimulating. (Fig. 10.)

TABLE II.—*Statement of results, experiment 2.*

Number of culture	1	2	3	4	5	6	7
Concentration:							
Before growth	0	$\frac{M}{500,000}$	$\frac{2\ M}{500,000}$	$\frac{4\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{10\ M}{500,000}$
After 7 days	$\frac{12\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{11\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{6\ M}{500,000}$
After 14 days	$\frac{12\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{11\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$

Number of culture	8	9	10	11	12	13	14
Concentration:							
Before growth	$\frac{12\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{22\ M}{500,000}$	$\frac{24\ M}{500,000}$
After 7 days	$\frac{5\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{11\ M}{500,000}$
After 14 days	$\frac{8\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{9\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{7\ M}{500,000}$	$\frac{7\ M}{500,000}$

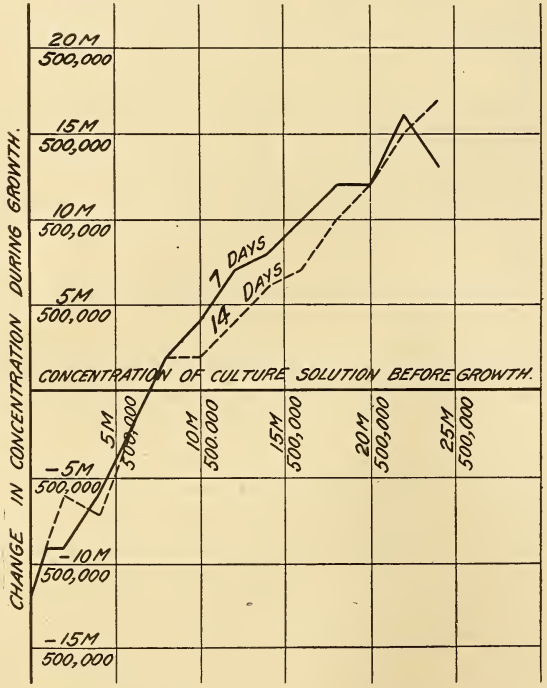

FIG. 10.—Curves showing relation of initial concentration of culture solutions to absorption and excretion of salts during growth in solutions of $Ca(NO_3)_2$ grading by increments of $\frac{2\ M}{500,000}$ from distilled water to $\frac{24\ M}{500,000}$.

EXPERIMENT 3.

Experiment 3 embraced a series of 14 solutions, of which 11 were of equivalent concentration at $\frac{M}{5{,}000}$ and graded by tenths from $\frac{10}{10}$ $Mg(NO_3)_2$ to $\frac{10}{10}$ $Ca(NO_3)_2$. Duration of cultures, 7 days; temperature 22° at the beginning and at the end.

TABLE III.—*Statement of results, experiment 3.*

No. of culture	1	2	3	4	5	6	7
Composition	1 Mg.	10 Mg.	9 Mg+1 Ca.	8 Mg+2 Ca.	7 Mg+3 Ca.	6 Mg+4 Ca.	5 Mg+5 Ca.
Concentration:							
Before growth.	$\frac{10\ M}{500,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$
After growth.	$\frac{9\ M}{500,000}$	$\frac{9\ M}{50,000}$	$\frac{7\ M}{50,000}$	$\frac{8\ M}{50,000}$	$\frac{6\ M}{50,000}$	$\frac{6\ M}{50,000}$	$\frac{5\ M}{50,000}$
No. of culture	8	9	10	11	12	13	14
Composition	4 Mg+6 Ca.	3 Mg+7 Ca.	2 Mg+8 Ca.	1 Mg+9 Ca.	10 Ca.	1 Ca.	Distilled water.
Concentration:							
Before growth.	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{50,000}$	$\frac{10\ M}{500,000}$	0
After growth.	$\frac{6\ M}{50,000}$	$\frac{5.5\ M}{50,000}$	$\frac{6.5\ M}{50,000}$	$\frac{6\ M}{50,000}$	$\frac{7\ M}{50,000}$	$\frac{10\ M}{500,000}$	$\frac{8\ M}{500,000}$

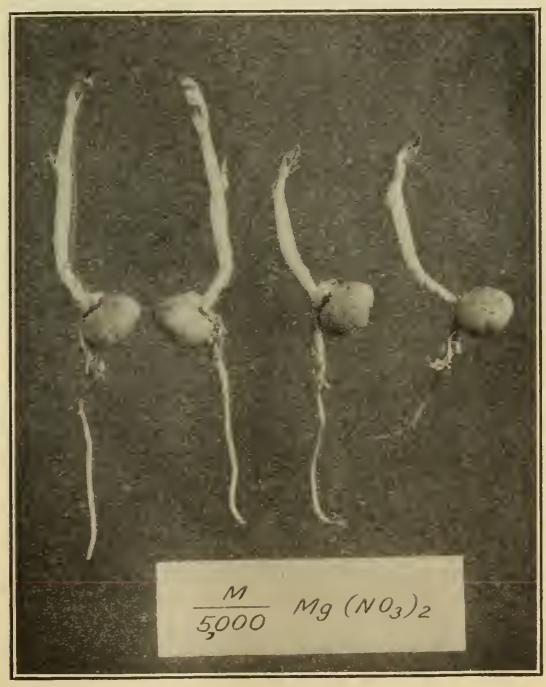

FIG. 11.—Seedlings of culture 2, experiment 3.

These results indicate (1) that in solutions (Nos. 2–12) 10 times as concentrated as solutions near equilibrium concentration (Nos. 1 and 13), a magnesium to calcium ratio of $\frac{9}{1}$ is as favorable to absorption as calcium alone; and (2) that the most efficient absorption takes place from solutions with magnesium and calcium present in approximately equivalent concentrations, i. e., in the ratio $\frac{5}{5}$. Since in this

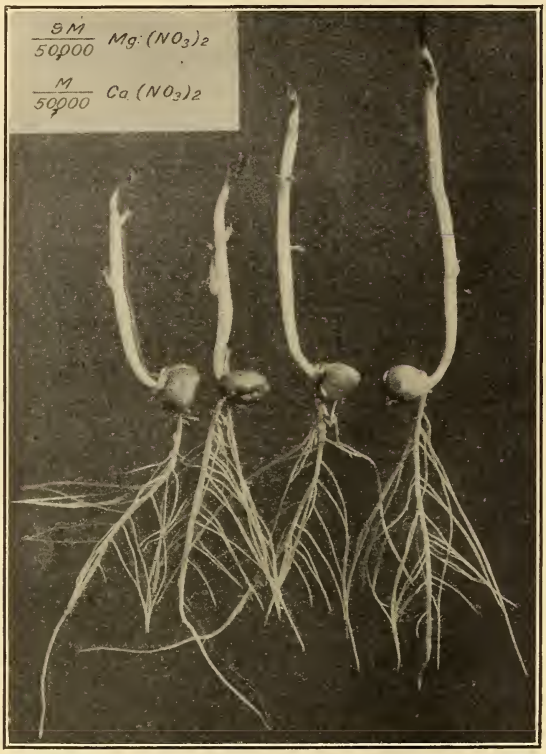

FIG. 12.—Seedlings of culture 3, experiment 3.

experiment the solutions of equivalent concentration were 10 times as dilute as in experiments A and B, it is interesting to note that the same ratio of magnesium to calcium as in those experiments sufficed to bring about perfect root development. In the figures illustrating the more significant cultures of this series note especially the contrast between the effects of $\frac{10\ M}{50{,}000}$Mg and $\frac{9\ M}{50{,}000}$Mg + $\frac{M}{50{,}000}$Ca (figs. 11 and 12; cf. figs. 1 and 8); the general similarity of cultures in $\frac{9\ M}{50{,}000}$

Mg + $\frac{M}{50,000}$Ca and in $\frac{10\ M}{50,000}$Ca (figs. 12 and 13); the slightly beneficial effect of $\frac{M}{50,000}$Ca and the markedly harmful effect of $\frac{M}{50,000}$ Mg, as contrasted with distilled water. (Figs. 14, 15, and 16.)

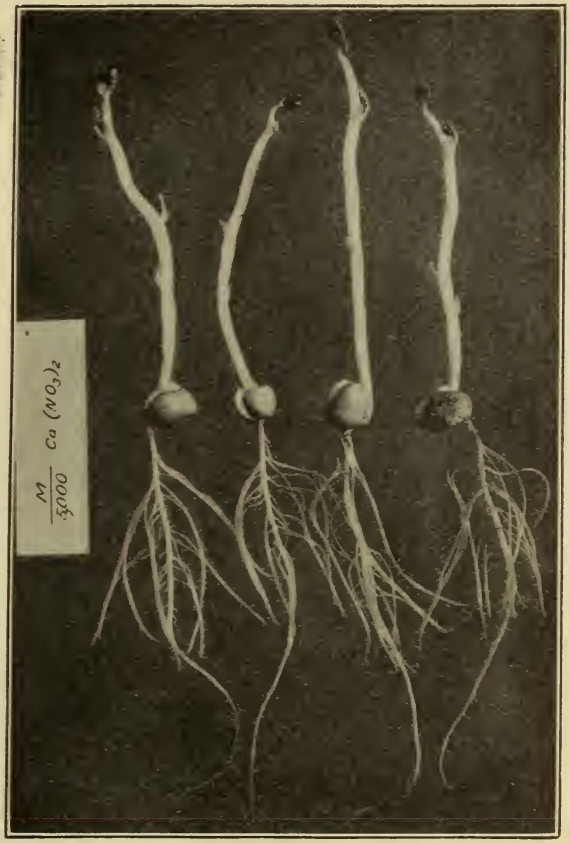

FIG. 13.—Seedlings of culture 12, experiment 3.

EXPERIMENT 4.

The series of culture solutions embraced in experiment 4 was identical with that of experiment B. Duration, 8 days; temperature at beginning and at end, 23.5°; stages of development as in experiment A. (Pl. I.)

TABLE IV.—*Statement of results, experiment 4.*

No. of culture	1	2	3	4	5	6	7
Composition.....	Mg.	10 Mg.	9 Mg+Ca.	8 Mg+2 Ca.	7 Mg+3 Ca.	6 Mg+4 Ca.	5 Mg+5 Ca.
Concentration:							
Before growth.	$\frac{100\ M}{500,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$
After growth.	$\frac{97\ M}{500,000}$	$\frac{97\ M}{50,000}$	$\frac{93\ M}{50,000}$	$\frac{91\ M}{50,000}$	$\frac{91\ M}{50,000}$	$\frac{90\ M}{50,000}$	$\frac{91\ M}{50,000}$

No. of culture	8	9	10	11	12	13	14
Composition.....	4 Mg+6 Ca.	3 Mg+7 Ca.	2 Mg+8 Ca.	Mg+9 Ca.	10 Ca.	Ca.	Distilled water.
Concentration:							
Before growth.	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{50,000}$	$\frac{100\ M}{500,000}$	0
After growth.	$\frac{90\ M}{50,000}$	$\frac{91\ M}{50,000}$	$\frac{91\ M}{50,000}$	$\frac{92\ M}{50,000}$	$\frac{94\ M}{50,000}$	$\frac{70\ M}{500,000}$	$\frac{17\ M}{500,000}$

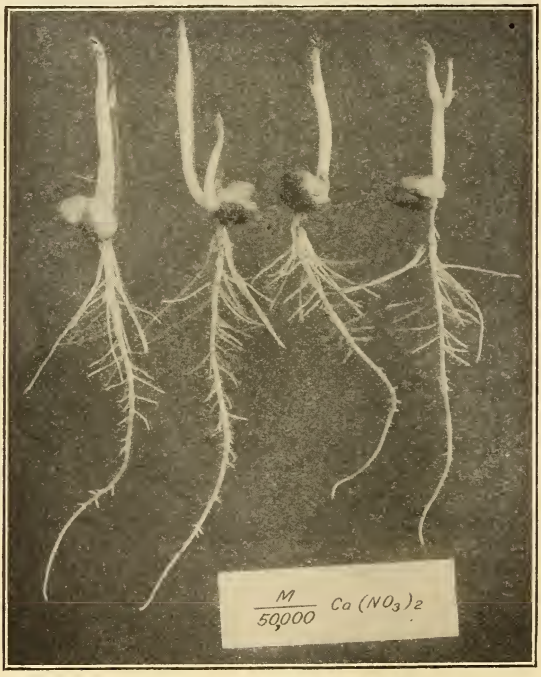

FIG. 14.—Seedlings of culture 13, experiment 3.

These results show, as did those of experiment 3, (1) that absorption from solutions containing magnesium and calcium in the ratio $\frac{9}{1}$ is about the same as from equivalent solutions of calcium alone; (2) that the most efficient absorption is from solutions containing mag-

nesium and calcium in the ratio $\frac{5}{5}$. It must also be noted that although the equivalent solutions (Nos. 2 to 12) were 10 times as concentrated in this series as in experiment 3 the absorption from each solution was only about twice as great.

FIG. 15.—Seedlings of culture 1, experiment 3.

EXPERIMENT 5.

In experiment 5 a series of solutions of equivalent concentration, but grading from 100 Mg to 78 Mg+22 Ca, was used. The concentration $\frac{M}{20,000}$ was chosen because it was less than the concentration which quite inhibited the growth of laterals, but was well above the equilibrium concentration. The ratios of magnesium to calcium are grouped closely about the significant ratio $\frac{9}{1}$ at which calcium

entirely overcomes the harmful effect on root growth of magnesium alone. The duration of cultures was eight days; temperature at the beginning 21.5°, at the end 19.5°.

The influence of calcium on root development in the cultures of this series was extremely interesting. In experiment C, as in experiment 5, calcium nitrate was substituted for magnesium nitrate by increments of a hundredth of the total salt concentration of the solution. In the former case, however, the salt concentration was

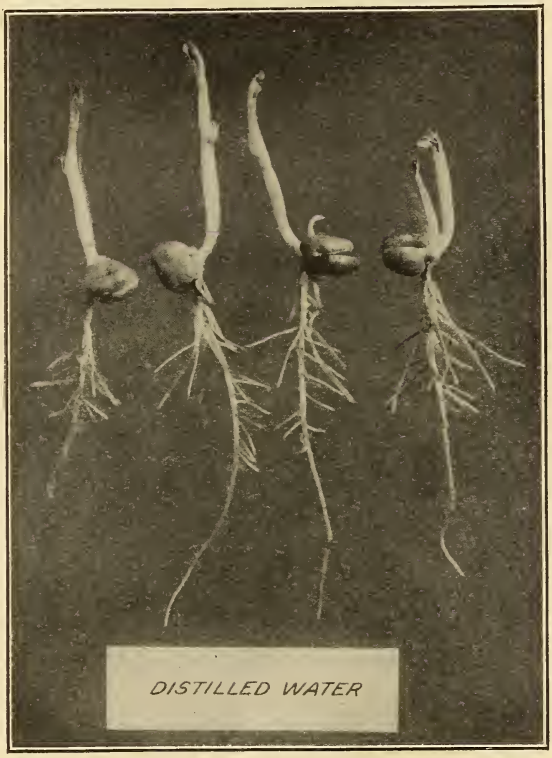

FIG. 16.—Seedlings of culture 14, experiment 3.

such that magnesium nitrate alone killed the roots, whereas in the latter case it was such that magnesium nitrate alone retarded the growth of lateral roots and did not kill the primary. In experiment C the lateral roots did not attain as perfect a development as in distilled water until the ratio of magnesium to calcium reached $\frac{92}{8}$. In experiment 5, on the other hand, the first increment of calcium (making the ratio of magnesium to calcium $\frac{99}{1}$) permitted laterals to develop as well as in distilled water. The concentration of calcium

nitrate which made the difference between roots with almost no laterals and roots with well-developed laterals was $\frac{M}{2{,}000{,}000}$, corresponding to one part, by weight, of calcium in 50,000,000 parts of water. The plants of the three significant cultures of this series are shown in figures 17, 18, and 19.

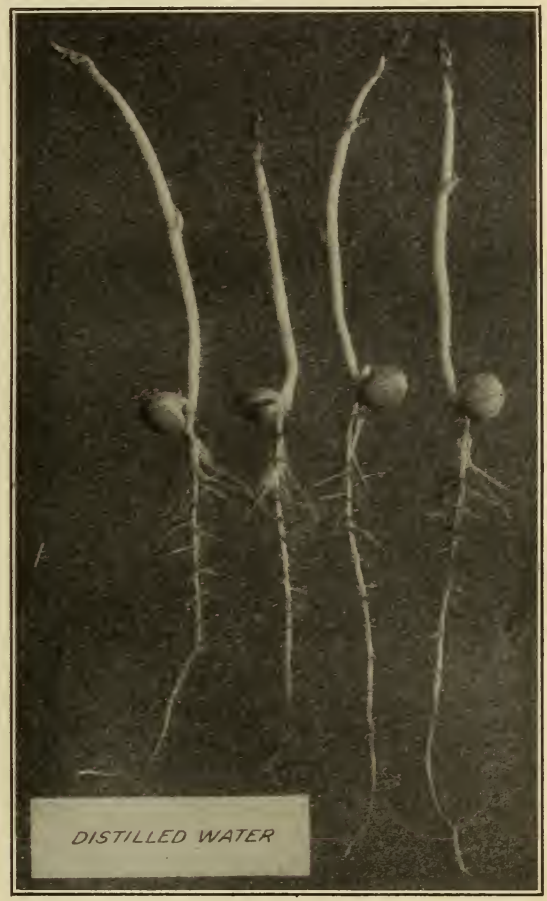

FIG. 17.—Seedlings of culture 14, experiment 5.

TABLE V.—*Statement of results, experiment 5.*

Number of culture	1	2	3	4	5
Composition	100 Mg.	99 Mg+1 Ca.	98 Mg+2 Ca.	96 Mg+4 Ca.	94 Mg+6 Ca.
Concentration:					
Before growth	$\frac{100\ M}{2{,}000{,}000}$	$\frac{100\ M}{2{,}000{,}000}$	$\frac{100\ M}{2{,}000{,}000}$	$\frac{100\ M}{2{,}000{,}000}$	$\frac{100\ M}{2{,}000{,}000}$
After growth	$\frac{43\ M}{2{,}000{,}000}$	$\frac{54\ M}{2{,}000{,}000}$	$\frac{28\ M}{2{,}000{,}000}$	$\frac{48\ M}{2{,}000{,}000}$	$\frac{40\ M}{2{,}000{,}000}$

TABLE V.—*Statement of results, experiment 5*—Continued.

Number of culture	6	7	8	9	10
Composition	92 Mg+8 Ca.	90 Mg+10 Ca.	88 Mg+12 Ca.	86 Mg+14 Ca.	84 Mg+16 Ca.
Concentration:					
Before growth	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$
After growth	$\frac{59\ M}{2,000,000}$	$\frac{33\ M}{2,000,000}$	$\frac{52\ M}{2,000,000}$	$\frac{50\ M}{2,000,000}$	$\frac{36\ M}{2,000,000}$

Number of culture	11	12	13	14
Composition	82 Mg+18 Ca.	80 Mg+20 Ca.	78 Mg+22 Ca.	Distilled water.
Concentration:				
Before growth	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$	$\frac{100\ M}{2,000,000}$	0
After growth	$\frac{61\ M}{2,000,000}$	$\frac{28\ M}{2,000,000}$	$\frac{46\ M}{2,000,000}$	$\frac{43\ M}{2,000,000}$

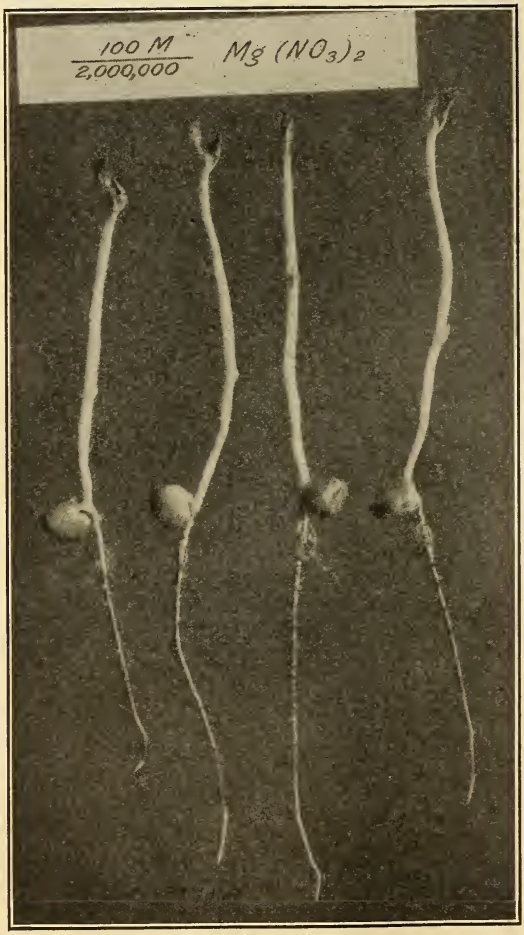

FIG. 18.—Seedlings of culture 1, experiment 5.

It is obvious that these results show no progressive efficiency of absorption with increasing calcium which is comparable to the great differences in absorption shown by cultures 2 and 3 of experiments

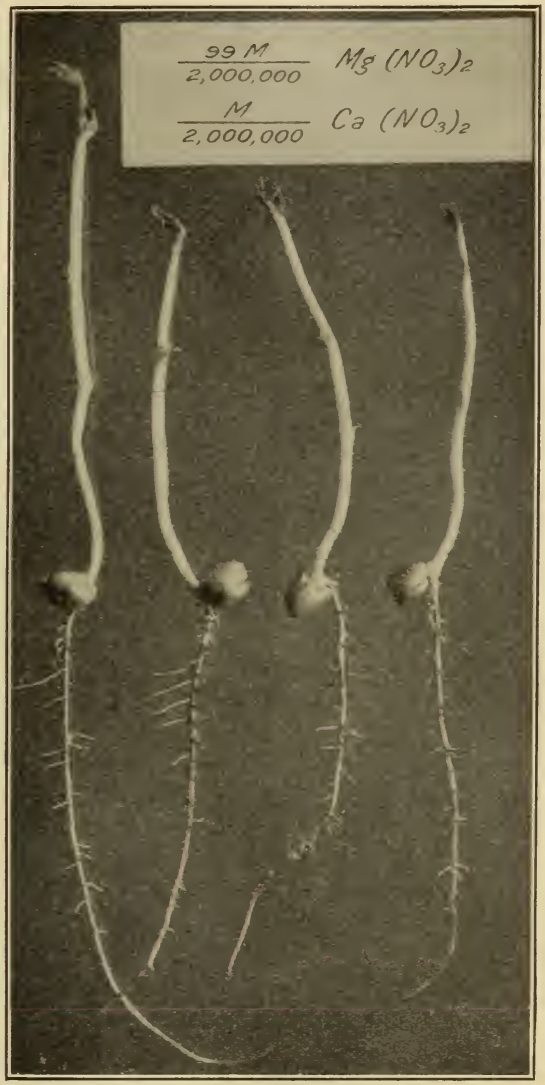

FIG. 19.—Seedlings of culture 2, experiment 5.

3 and 4. In experiments 3 and 4 what absorption there was from the magnesium solutions had to take place through the surface of an unbranched primary root of constantly diminishing vitality. In this experiment, on the contrary, the dilution of the magnesium

solution was such that lateral roots developed, providing an absorbing system at least comparable in extent and efficiency with the root systems in the other solutions.

The mean concentration of all the equivalent solutions after growth was $\frac{44\ M}{2{,}000{,}000}$. The distilled water culture had a concentration after growth of $\frac{43\ M}{2{,}000{,}000}$. Equilibrium concentration for magnesium alone (experiment 1) was $\frac{48\ M}{2{,}000{,}000}$; for calcium alone (experiment 2) $\frac{28\ M}{2{,}000{,}000}$. It will be noticed that in no case were culture solutions in experiment 5 exhausted beyond the equilibrium concentration for calcium alone.

EXPERIMENT 6.

Experiment 6 included a series of equivalent solutions of the concentration $\frac{75\ M}{500{,}000}$, varying from 75 Mg to 65 Mg+10 Ca. The duration was nine days; temperature at beginning 20.5°, at end 19°.

TABLE VI.—*Statement of results, experiment 6.*

Number of culture	1	2	3	4	5
Composition	10 Mg.	75 Mg.	74 Mg+1 Ca.	73 Mg+2 Ca.	72 Mg+3 Ca.
Concentration:					
Before growth	$\frac{10\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$
After growth	$\frac{16\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{43\ M}{500,000}$	$\frac{42\ M}{500,000}$	$\frac{60\ M}{500,000}$
Number of culture	6	7	8	9	10
Composition	71 Mg+4 Ca.	70 Mg+5 Ca.	69 Mg+6 Ca.	68 Mg+7 Ca.	67 Mg+8 Ca.
Concentration:					
Before growth	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$
After growth	$\frac{51\ M}{500,000}$	$\frac{37\ M}{500,000}$	$\frac{37\ M}{500,000}$	$\frac{27\ M}{500,000}$	$\frac{39\ M}{500,000}$
Number of culture	11	12	13	14	15
Composition	66 Mg+9 Ca.	65 Mg+10 Ca.	10 Ca.	1 Ca.	Distilled water.
Concentration:					
Before growth	$\frac{75\ M}{500,000}$	$\frac{75\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{M}{500,000}$	0
After growth	$\frac{23\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{23\ M}{500,000}$

The series of results of experiment 6 is in striking contrast to those of experiment 5. In experiment 5 the concentration of the equivalent solutions was so low that even the absorption from a solution containing only magnesium was sufficient to reduce it to equilibrium concentration. In experiment 6, on the other hand, tripling the concentration of the equivalent solutions resulted in showing the marked influence of successive increments of calcium on absorption. In culture 2 (magnesium alone) there were no lateral roots, and the nonfunctioning primary root did not alter the concentration of the solution. In culture 3, however, the substitution of one seventy-fifth of the magnesium by calcium resulted in the growth of laterals 1 to 4 mm. long, accompanied by appreciable absorption. The equivalent cultures from No. 6 on showed more perfect root systems, correlated with increasingly greater absorption.

EXPERIMENT 7.

A series of solutions containing magnesium and calcium in the molecular ratio $\frac{9}{1}$ were used in experiment 7; i. e., solutions containing enough calcium to insure perfect root development, but not enough to bring about maximum salt absorption. The duration of cultures was 7 days; temperature at beginning and at end of experiment 22.3°.

TABLE VII.—*Statement of results, experiment 7.*

Number of culture	1	2	3	4	5	6	7
Concentration:							
Before growth	Distilled water.	$\frac{M}{500,000}$	$\frac{2\ M}{500,000}$	$\frac{4\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{10\ M}{500,000}$
After growth	$\frac{12\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{9\ M}{500,000}$
Number of culture	8	9	10	11	12	13	14
Concentration:							
Before growth	$\frac{12\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{22\ M}{500,000}$	$\frac{24\ M}{500,000}$
After growth	$\frac{9\ M}{500,000}$	$\frac{11\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{15\ M}{500,000}$

These results (graphically represented in fig. 20) are directly comparable with those of experiments 1 and 2. As would have been expected, the equilibrium concentration $\frac{8\ M}{500,000}$ lies between that of magnesium alone $\frac{12\ M}{500,000}$ and that of calcium alone $\frac{7\ M}{500,000}$. With regard to the amounts of salt excreted into cul-

ture solutions below the equilibrium concentration and absorbed from solutions above this concentration, the results are very similar to those obtained with calcium alone (cf. experiment 2).

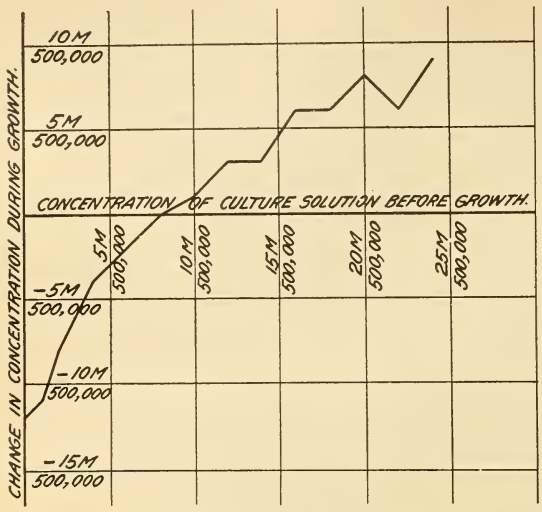

FIG. 20.—Curve showing relation of initial concentration of the culture solutions to subsequent excretion and absorption of salts by pea roots in solutions of $Mg(NO_3)_2+Ca(NO_3)_2$ in the molecular ratio $\frac{9}{1}$.

EXPERIMENT 8.

Solutions used in experiment 8 contained magnesium and calcium in the molecular ratio $\frac{5}{5}$, i. e., the ratio indicated in experiments 3 and 4 as the most favorable for absorption. Concentrations were determined after 6, 10, and 13 days. The temperature at the beginning was 22.3°; on successive dates when concentrations were determined, 22.3°, 22.1°, and 23.5°.

TABLE VIII.—*Statement of results, experiment 8.*

Number of culture	1	2	3	4	5	6	7
Concentration.							
Before growth	Distilled water.	$\frac{M}{500,000}$	$\frac{2\ M}{500,000}$	$\frac{4\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{8\ M}{500,000}$	$\frac{10\ M}{500,000}$
After 6 days	$\frac{10\ M}{500,000}$	$\frac{9\ M}{500,000}$	$\frac{9\ M}{500,000}$	$\frac{7\ M}{500,000}$	$\frac{5\ M}{500,000}$	$\frac{4\ M}{500,000}$	$\frac{2.5\ M}{500,000}$
After 10 days	$\frac{17\ M}{500,000}$	$\frac{22\ M}{500,000}$	$\frac{17\ M}{500,000}$	$\frac{15\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{13\ M}{500,000}$
After 13 days	$\frac{20\ M}{500,000}$	$\frac{24\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{19\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{19\ M}{500,000}$	$\frac{19\ M}{500,000}$
Number of culture	8	9	10	11	12	13	14
Concentration:							
Before growth	$\frac{12\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{16\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{20\ M}{500,000}$	$\frac{22\ M}{500,000}$	$\frac{24\ M}{500,000}$
After 6 days	$\frac{4\ M}{500,000}$	$\frac{2.5\ M}{500,000}$	$\frac{2\ M}{500,000}$	$\frac{2\ M}{500,000}$	$\frac{1.5\ M}{500,000}$	$\frac{3\ M}{500,000}$	$\frac{3\ M}{500,000}$
After 10 days	$\frac{12\ M}{500,000}$	$\frac{12\ M}{500,000}$	$\frac{10\ M}{500,000}$	$\frac{5.5\ M}{500,000}$	$\frac{6\ M}{500,000}$	$\frac{4.5\ M}{500,000}$	$\frac{4.5\ M}{500,000}$
After 13 days	$\frac{19\ M}{500,000}$	$\frac{18\ M}{500,000}$	$\frac{18.5\ M}{500,000}$	$\frac{14\ M}{500,000}$	$\frac{14.5\ M}{500,000}$	$\frac{11\ M}{500,000}$	$\frac{9\ M}{500,000}$

The plants of this experiment developed very rapidly, and had reached a state of maximum root development after six days. At this time the equilibrium concentration was about $\frac{5\,M}{500{,}000}$, being less than that found for solutions of magnesium or calcium alone, or for magnesium and calcium in the molecular ratio $\frac{9}{1}$. The absorption efficiency was so great that all solutions which at the start were above equilibrium concentration were brought below it before the rate of excretion became greater than the rate of absorption.[1] This was not the case in any other experiment. In figure 21 there are three curves. The one which represents the solutions at the end of six days gives the true equilibrium concentration, for up to that time the roots had been actively growing. The other curves merely indicate the rate at which the salt content of roots which are no longer actively growing passes into the culture solutions.

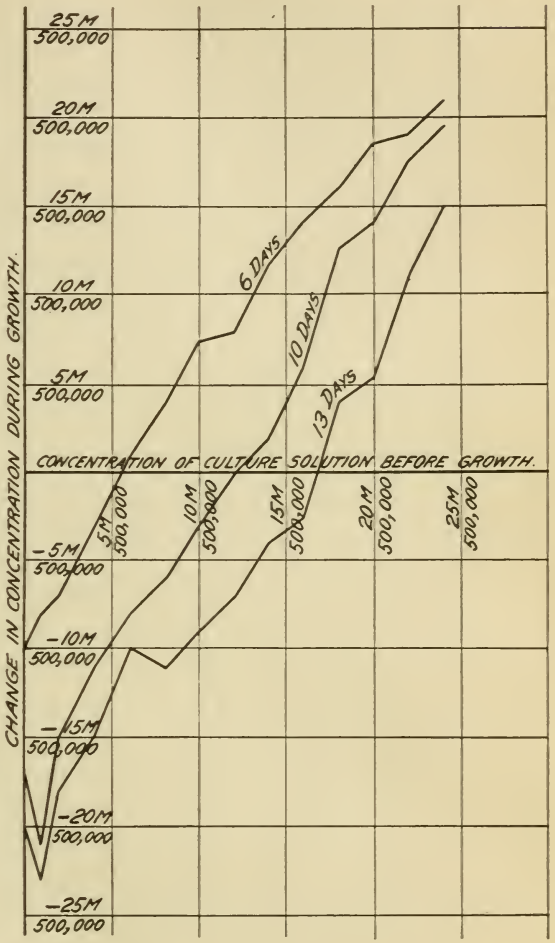

FIG. 21.—Curves showing relation of initial concentration of the culture solutions to subsequent excretion and absorption of salts by pea roots in solutions of $Mg(NO_3)_2+Ca(NO_3)_2$ in the molecular ratio $\frac{9}{1}$.

CONCLUSIONS.

Our work on the relation of roots to dilute solutions of calcium and magnesium nitrates has thus far shown: (1) That there is a definite

[1] It is obvious that in every culture whose development is limited by lack of light, the entire salt content of the root system must ultimately, as vitality wanes, pass into the culture solution. With peas, the rate of outgo exceeds the rate of intake as soon as root growth stops, long before the roots die or the cotyledons are exhausted.

concentration for each salt or mixture of salts at which the roots of peas absorb and excrete electrolytes at the same rate; (2) that if a culture solution is initially less concentrated than this equilibrium concentration, excretion from the roots overbalances absorption; (3) that if a solution is initially more concentrated than this equilibrium, absorption overbalances excretion; (4) that absorption from solutions initially above equilibrium concentration may carry them far below this concentration; (5) that the extent to which pea roots can carry the concentration of a solution below equilibrium concentration depends upon the ratio of magnesium to calcium; (6) that the molecular ratio which favors maximum absorption is $\frac{1}{1}$; (7) that the ratio of magnesium to calcium which insures good development of pea roots is $\frac{9}{1}$, if the solutions are so concentrated that their magnesium content alone would inhibit the development of lateral roots; but (8) that this ratio is nearer $\frac{99}{1}$ if the solutions are so dilute that the magnesium content alone would not inhibit the development of lateral roots.