STUDIES ON THE ABSORPTION OF AMMONIA AND NITRATE BY THE ROOT OF ZEA MAYS-SEEDLINGS, IN RELATION TO THE CONCENTRATION AND THE ACTUAL ACIDITY OF CULTURE SOLUTION

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With Plate I and 26 Text-figures

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Introduction

Up to the present day, investigation on the intake of salts by plants has been performed along two lines. The first method is to investigate the growth by means of sand and water cultures. The results of these investigations are of great importance, because they furnish some fundamental conceptions with respect to plant nutrition. By ash analyses, we come to know which salt is taken in great quantity and which salt is indispensable for the normal growth of plant. But what is called an essential element for plant nutrition is deduced from the results of growth experiments. How is the electrolyte absorbed? Which factors govern the process of absorption? About these problems, the results of sand and water culture experiments only can tell us nothing with certainty. The nutrient value of a salt firstly depends upon the absorption of plants, and this is certainly different among different kinds of plants. Thus a salt may be valuable for one plant, but valueless for another. Above all, good growth is not necessarily a result of great absorption and cannot be regarded as an indicator of absorption.

In the second line of investigation, the problem is concerned with the mechanism of the cell's absorption of dissolved substances. We have numerous theories on permeability based on the observations of different material using differing methods. In spite of numerous theories, the mechanism of absorption of salts is by no means clear. Most observations of this kind in plant physiology based on experiments with isolated cells or tis-

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sues, and in most cases the permeability was measured by the method of deplasmolysis. That permeability in these conditions does not occur normally has been proved by the recent investigations, and the theories elaborated from results of these observations can not be applied to the absorption of salts in dilute solutions by the root system of higher plants. As has been pointed out by STILES (1624a), these date may be of interest, but "we can hardly expect to be able to formulate the laws governing the passage of substances into and out from the cells without adequate quantitative data."

In short, our knowledge regarding the absorption of salts by plants is far from satisfactory. To attack the problem in the quantitative way is certainly one of the good methods for understanding the nature of salt absorption. In this connection, the recent researches of HOAGLAND and his coworkers (1928) are of special interest. By the method of microanalysis, they found many interesting phenomena regarding the accumulation of ions in the cells of *Nitella*.

But investigations on the absorption of salts by the root system of higher plants are rather few(1). From 1927 to 1929, the writer has carried out a series of experiments dealing with the absorption of ammonia and nitrate from ammonium nitrate by the root system of Zea Mays and a few other plants. This salt was chosen for study, because the cation and anion of this salt contain equimolar nitrogen and can be determined microanalytically with reliable accuracy.

Method in general

All experiments were carried out in water culture. Since the technique has been described in an earlier paper, it needs not to be repeated here.

As culture plants, mostly seedlings of sugar corn (Golden Bantam) were used throughout the studies. The seeds were immersed over night in tap water, then were sown in saw dust for germination. When the shoot was about 1 or 2 cm. in length, and its lateral roots had not yet appeared, the seedlings were taken out from the germination box, rinsed with tap water, and set up for preliminary culture in order to secure the satisfactory growth of the root system(2). The culture solution was a mixture of calcium chloride and potassium chloride with a concentration of 0.002 M. The mixture was found particularly suitable for the growth of a root system:

(1) The literatures regarding this problem will be found in following pages.

(2) Unless otherwise noted, the residual endosperm was not picked off.

a duration of a few days, usually five or seven, was sufficient to secure a root system good enough to satisfy the requirements of the experiment. The root system thus obtained was long and fine, and snow white in color. In the later part of the work, tap water was used in the preliminary culture instead of the mixture; the result was also excellent.

When the root system reached the desired state of growth, the seedlings were taken out together with the cover of the culture vessel, washed thoroughly with distilled water, and then replaced in the culture solution for study of absorption. At the end of the experiment, the culture solution was made up to the original volume and amount of ammonia and nitrate absorbed was analysed. In this case, the root system should be carefully washed with distilled water, since the solutes may adhere to the surface of the roots. In general, the amount of water lost by transpiration within twenty four hours was about 20-30 cc., this supplementary volume of water was sufficient for the washing of the roots. The seedlings were dried in a dry oven at 75–80°C. and the dry weight of shoot and root determined separately.

Determination of ammonia: Ammonia was estimated by the micro-Kjehedahl method. A suitable amount of the sample was placed in a 100 cc. long-necked, round-bottomed micro-Kjehedahl-flask and 2–6 cc. of standard hydrochloric acid (0.01 N) was placed in a 100 cc. receiving flask, using a 2 cc. micro-burette graduated to 0.01 cc. and two drops of 0.1 percent sodium alizarinsulphonate were added as indicator. Five cc. of saturated caustic potash were added to the sample through a separate funnel. If the sample exceeds 20 cc., it is desired to heat the micro-Kjehedahl flask with a burner till boiling, in order to hasten the distillation of ammonia. But when the sample was less than 20 cc., this heating was not necessary. When the heating was strong and steam powerful, the distillation usually lasted ten minutes. At the end of distillation the receiving flask must be lowered a little, allowing the outlet tube of the cooler to extend above the surface of the acid, so that the inner side of the outlet tube may be rinsed by the water stream. The last operation is of great importance: if the apparatus is disconnected before the lowering of the receiving flask, part of the liquid in the receiving flask would remain in the tube. Moreover, a sudden stoppage of distillation always causes a backward flow. Therefore, it is advisable to lower the receiving flask and let the water stream pass through the tube usually for two or three minutes, then wash the outside of the tube with distilled water. When distillation was complete, the excess of HCl was titrated with 0.01 N NaOH, using a 2 cc. microburette graduated to 0.01 cc. The standard alkali solution was carefully prepared according to the direction of CLARK (1923, p. 101), and stored in a non-alkaline glass bottle, to which a microburette, made of glass of the same quality, and soda-lime guard tubes were attached.

Determination of nitrate: Nitrate was estimated colorimetrically by the phenol disulphonic acid method. The procedure was as follows: a certain volume of the sample which usually contained one cc. of about 0.002 N of nitrate was taken in an evaporating dish and evaporated in the water bath. One cc. of phenol disulphonic acid(1) was added to it and the mixture was thoroughly stirred with a glass rod. Ten minutes later, 10 cc. of distilled water were added to it, stirred with a glass rod and the mixture was made alkaline by adding 5 cc. of concentrated ammonia water. Thus appeared a yellow colour which resembles that of picric acid. The whole was poured into a Ukena's colorimeter, which is a glass tube of 30 cc. volume and graduated to 0.1 cc. Then the volume was made up to 30 cc. The colour was compared with that of the standard, which contained one cc. of 0.001 N KNO_3. Since the amount of nitrate in the sample is always greater than that in the standard, half of the volume should be thrown away. Distilled water was added till the colour became identical with that of the standard. If the first throwing away was not sufficient, the volume was made up to 30 cc. again and another half discarded; then distilled water was added again till the colour was exactly identical. The amount of nitrate was calculated according to the follwing formula:

$$\frac{a \times 2^x}{30 \times C} = \text{Concentration of } NO_3 \text{ in normal}$$

a .... reading in cc. when the colour is identical to that of the standard.

x .... number of times of discarding.

C .... amount of sample taken in cc.

In estimation of nitrate, the following treatment is of great importance. (1) When the sample contains ammonia or its salts, it must be driven out by adding a suitable amount of NaOH during the evaporation. (2) When the sample contains chlorine or halogen compounds, they must be excluded with silver sulphate. The necessity of excluding chloride in the estimation of nitrate by this method has been reported by many authors (HARPER, 1924). But, as far as the writer knows, no one has noticed

(1) This reagent was prepared by heating the mixture of 37 gm. of pure sulphuric acid and 3 gm. of carbolic acid in the water bath during 6 hours.

the importance of excluding bromide, iodide and other halogen compounds. During the course of this work, the writer found that the existence of bromide and iodide in a solution have the same effect on the estimation of nitrate as chloride, and that they can be removed by the same treatment. In this case, the solid silver sulphate should be used in order to prevent any change of volume. The precipitate of silver chloride was separated by centrifuging, or the sample was left over night and the clear upper part was taken out with a pipette. A few cc. of silver chloride were added to a part of the clear liquid in order to test again the existence of chlorides or other halogen compounds. That the silver sulphate used should be free from nitrate is a matter of course. It must be tested before use. In our case, Kahlbaum's silver sulphate was used throughout the work. (3) The colour of the standard changes gradually. Therefore it should be renewed at least once a week.

Estimation of pH: The hydrogen ion concentration of the solution was determined colorimetrically by the method of CLARK and LUB (CLARK, 1925), using their indicators and standards.

The culture vessels used in the present work, with few exceptions, were porcelain cylinders of capacity of 400 cc. and 350 cc. In the later part of work, cylinders of 400 cc. were exclusively used in preliminary culture. These cylinders were specially made for the purpose of this work and had been tested as to be free from any alkaline matter.

In order to secure distilled water free from oligodynamic substance; the common distilled water was treated with Kahlbaum's blood charcoal, shaken thoroughly, stood still for at least twelve hours and then filtered several times. The reaction of the distilled water thus prepared was a little alkaline, when carbon dioxide was driven out: the pH-value was about 7.6–8.0. According to MICHAELIS, blood charcoal is an "Ampholytoid" which adsorbs $H^{\cdot}$ in acid and OH' in an alkaline solution. As the result of this "Neutralisationseffekt" of the blood charcoal, the reaction of the solution always changes to the neutral point (MICHAELIS, 1922). KROETZ (1924) found that the isoelectric point of animal charcoal is pH 7.58. Though the blood charcoal used in this work was different from that of MICHAELIS and KROETZ, the slight alkalinity of the distilled water treated with it may have some relation to this ampholytic behaviour of the blood charcoal. On the other side, the filter paper used was responsible to some extent for the alkalinity of the distilled water. If one places a piece of filter paper in an ERLENMAYER flask with 50 cc. of redistilled water for one night, the reaction of the redistilled water would shift from pH 6.9

to 7.8 when carbon dioxide was driven out. The effect of filter paper on the reaction of distilled water and salt solutions has been found by many authors. ATKINS (1924) found that the reaction of soil solution was changes by filtering. TAMIYA and ISHIUCHI (1926) found that the acidity of distilled water and salt solution was changed by adding filter paper and they ascribed the cause of this chage to the adsorption of cations by cellulose. Recently GUSTAFSON (1928) recognized the same fact as ATKINS and suggested that in clearing the soil solution from the suspended particles for pH-determination, centrifuging is better than filtering. The results of TAMIYA and ISHIUCHI mentioned above show that filter paper also acts as an ampholytoide. In our case, the neutral redistilled water always becomes alkaline within the lapse of 24 hours by shaking it with filter paper. We have to concluded that the impurity (ash content?) of the filter paper has something to do with the alkalinity of the distilled water. Anyhow, the distilled water thus treated was free from copper and other origodynamically toxic substances. *Spirogyra* could grow in it quite well for one day or so, while the untreated distilled water is decidedly toxic to that algae (SAKAMURA, 1922).

In preparing the standard solution of acid and alkali and in the analysis of ammonia redistilled water was used.

In the present work, the analytical results were expressed in normality of ammonium and nitrate ions. This is the most right way for comparing the relative absorption of cation and anion[(1)]. Moreover it is one of our objects to see whether the unequal absorption of cation and anion of the salts used as source of nitrogen has any relation to the change of reaction of the culture medium. The difference in normality of cation and anion of the culture solution measured after the absorption is important for our purpose.

In comparing the rate of absorption, milligrams of nitrogen per one gram of dry weight of roots were taken as the unit. For the absorption, area of root surface rather than the dry weight is responsible. But since there is no suitable method for measuring the area of the root, the dry weight of root system was adopted for convenience. That this answered the purpose very well is obvious from the results of the following experiment. This experiment was carried out in order to learn the probable

(1) In this paper, the term "absorption of cation or anion" was sometimes used to indicate the absorption of the basic or acidic part of a salt; it does not always mean that ion is absorbed as such, since we do not yet know whether salt is absorbed in the form of ion or molecule.

error of the determination of absorption which is inevitable when a different set of plants is used in each analysis. Four series of culture with differings number of seedlings varied from 3 to 6 were grown in 0.005 N NH_4NO_3-solution for 48 hours. Each culture contained 350 cc. of solution.

Series No.	Number of seedlings	NH_4 absorbed (0.00001 N)	Dry weight of root (gm)	Nitrogen absorbed per 1 gm. of dryroot (mg)
1	3	81	0.0946	42.2
	3	81	0.0935	42.5
2	4	94	0.1100	41.9
	4	86	0.1000	42.1
3	5	123	0.1415	42.5
	5	121	0.1400	42.4
4	6	136	0.1610	41.5
	6	131	0.1520	42.2

The amount of nitrogen absorbed per one gram of dry weight is fairly equal amongst the eight cultures. The probable error is ± 0.25 mg.

The absorption of ammonia and nitrate in relation to the concentration of culture solution

Studies on the influence of concentration of culture medium upon the absorption of nutrient salts by plants are rather few. SCHREINER and SKINNER (1910) were able to find out an optimum concentration for the absorption of some important nutrient ions such as NO_3, PO_4, K and NH_4. POUGET and CHOUCHAK (1910–1912) recognized the same fact. According to TRUE and BARTLETT (1912), there is a certain limit of concentration of Ca $(NO_3)_2$ where the absorption and excretion of ions comes to an equilibrium; above this concentration, absorption predominated, below this point, excretion took place. NATHANSOHN (1904) found that the tuber tissue ot Dahlias takes in a certain amount of inorganic salts, and that amount of absorption has very much to do with the concentration of salts both inside and outside of the plant cell. Similar results have been obtained by MEUER (1909) and TRUBETSKOVA (1927). Most of these authors regard the salts absorbed always as a whole. On the other hand, a few authors have observed the effect of concentration of the culture solution on the relative absorption of cation and anion of a salt. (NATHANSOHN, 1904;

MEIJER, 1909; PANTANELLI, 1915; REDFERN, 1922; STILES, 1924; PRIANISCHNIKOW, 1927; DAVIDSON, 1927; NIKLEWSKI, KRAUSE and LEMANCZYK, 1928). Their results show that the more dilute the solution, the less the divergence between the absorption of cation and anion.

Early in 1927, the writer began to work out a series of experiments dealing with the effect of concentration of culture medium upon the relative absorption of ammonia and nitrate in a solution containing ammonium nitrate as the source of nitrogen. The effect of concentration means the effect of the concentration (1) of the culture solution as a whole; (2) of source of nitrogen when the concentration of other salts was constant and (3) of other salts except the source of nitrogen. Besides the above mentioned, a series oxperiments dealing with single NH_4NO_3 solution was also carried out.

Seedlings of maize were exclusively used as culture plants. After preliminary culture, the seedlings uniformly developed were transferred to the culture solution and grown in the green house for about two weeks. During this period distilled water was added from time to time to prevent the change of concentration due to the loss of water by transpiration. Harvesting was practiced several times a week and the amount of ammonia and nitrate absorbed was determined by analysis of the external solution.

The stock solution used in this series of experiments were as follows:

A.

KH_2PO_4	2.5	gm.
$MgSO_4.7\ H_2O$	,,	,,
$CaCl_2.2\ H_2O$	0.97	,,
Distill. Water	500	cc.

B.

NH_4NO_3	3.92	gm.
Distill. Water	200	cc.

Iron was added in the form of $FeCl_3$, two drops of a 2 % solution were added to each litre of culture solution at the commencement of experiment.

A. Complete solution with varied concentrations

Five experiment were carried out to study the influence of the varied concentration of the complete solution upon the absorption of ammonia and nitrate. The composition of the culture solution was as follows:

No.	Stock solution		H_2O cc.	Sum cc.	Concentration of NH_4NO_3 (N)	pH
	A	B				
1	300	300	2400	3000	0.02400	4.6
2	150	150	2700	,,	0.01215	5.0
3	45	45	2910	,,	0.00354	5.0
4	15	15	2970	,,	0.00122	5.4
5	1.5	1.5	2997	,,	0.00012	5.6

Three seedlings were grown in each culture containing 300 cc. of nutrient solution. The concentration of ammonium nitrate showed in these cultures was all found as the result of analyses.

Experiment 1

NH_4NO_3=0.02400 N.

Perhaps due to the high concentration of the culture solution, the growth of the seedlings was by no means favourable. Hypertropyhy of the lateral root took place as the experiment went on, and the whole root began to decay on the ninth day after the beginning of the experiment. The root surface was surrounded by mycelium of filamentous fungi, but the inner tissue was healthy as it was not stained by cotton blue.

The analytical results of this experiment are shown in Table 1. It is worthy to note that very small amount of nitrate was absorbed by the root when compared to the absorption of ammonia. At the first harvest (four days after the beginning of the experiment), no nitric nitrogen was absorbed at all, while almost 50 mg. of ammoniacal nitrogen were taken in by the roots. The difference of ammonia and nitrate absorbed was 0.00177 N and this difference became greater and greater as the experiment went on.

The unequal absorption of ammonia and nitrate resulted in the development of high acidity in culture solution. In general, the change of pH-value of the culture solution was proportional to the difference between ammonia and nitrate absorbed, it shifted from pH 4.6 to 3.1 within four days. This sudden increase of acidity may be the cause of the unfavourable growth and the hypertrophy of the lateral roots.

From the ninth day on, practically no appreciable amount of nitrogen was absorbed. On the contrary, a small part of nitrate was given off from the roots. As described above, the root system began to decay on the ninth day, it might be expected that a part of nitrate which had accumulated in the root tissue, but had not been utilized by the plant, would return to the external solution. Whether this hindrance of absorption was directly due to high acidity or indirectly due to the unfavourable growth of the roots which was apparently a result of great concentration of the hydrogen ions of the culture solution is a problem which is too difficult to be solved with a single experiment. Perhaps both were the causes. As will be seen later, both the growth of root and the absorption of nitrogen were hindered by high acidity of the culture solution.

At any rate, in such concentrated solutions the root system absorbed more ammonia than nitrate. The absorption ratio NH_3/NO_3 was never less than 3. In other words, the root system of maize absorbed ammonia three times greater in amount than nitrate. As the result of this unequal absorption, the reaction of the culture solution became suddenly acidic and the root growth and absorption of nutrients were hindered.

Experiment 2

NH_4NO_3=0.01215 N.

The concentration of this solution is equal to that of KNOP solution. The plants grown in it were healthy and excellent till the 15th day when the root system began to decay. At that time the pH-value of the culture solution became 2.7.

Table 2 shows the result of this experiment. It is interesting to see that by this concentration too, no nitrate was absorbed by the root at the beginning of experiment. Ammonia was chiefly absorbed at first, and after ammonia was absorbed to some extent, the absorption of nitrate began. Just in the case of the foregoing experiment, the reaction of the culture solution changed suddenly from pH 5.0 to pH 3.8. The absorption ratio NH_3/NO_3 was greater than 2 during the whole experiment.

The results of this experiment resemble that of the foregoing one in every respect. The only difference is that in the case of Experiment 1, the pH-value shifted to 3.1 suddenly and this high acidity caused the hindrance of growth and absorption, while in the case of the present experiment, the difference of cation and anion absorbed did not exceed 0.001 N in the first week and the pH-value did not become less than 3.4, so that both growth and absorption took place in the next week. In the later half of the experiment duration, the culture plants got twice weight of dry substance and absorbed thrice amount of nitrogen as in the first half.

Perhaps a culture solution having a pH-value equal to 3.4 is not extremely poisonous to the growth of seedling of maize.

Another notable point is that in the later part of the experiment, the amount of nitrate ions absorbed was comparatively greater than in the first half, so that the absorption ratio became smaller and smaller. This phenomenon can be easily explained when one considers the fact that nitrate is better absorbed by the root of higher plant in acid condition; a fact wich was found by THERON (1923) and PRIANISCHNIKOW (1926) and which is also confirmed by the writer, as will be seen in the later part of the present work.

Experiment 3

NH_4NO_3=0.00354 N.

The vessels used in this experiment were cylinders of Jena glass, having a capacity of 300 cc. The growth of seedlings was very excellent, much better than when grown in the more concentrated solution of the foregoing experiments. However, the colour of the root system became pink after the 13th day, showing the first symptom of decay. The chief cause of this symptom is the scarcity of nitrogen. As will be seen from Tableg 3, almost all the nitrogen in the solution was absorbed by the root before the 13th day.

Contrary to the results of the foregoing experiment, the nitric nitrogen of this solution was comparatively better absorbed by the roots. Except for the first 24 hours, the absorption ratio NH_3/NO_3 was usually less than two, and after the 12th day it became practically the same, a fact which could not be seen in the case of more concentrated solutions.

In spite of this small absorption ratio, the difference between cations and anions absorbed was quite great. Accordingly, the acidity of the culture solution changed from pH 5.0 to 3.8 in the first 24 hours and became greater and greater as the experiment went on till on the 12th day when all the amount of ammonia in the solution being exhausted, it became less acidic. Notwithstanding the high acidity, the seedlings were grown in it very well. This is a result which is inconsistant with that of the first experiment. This inconsistancy may be explained in two ways. Firstly, two solution of the same pH-value but of different salt concentration may have a different influence on the culture plants; the more concentrated the solution, the more unfavourable is the influence. This statement is self-evident. Secondly, though nitrate can be better absorbed in an acid solution, the high concentration of the culture solution in the first experiment rendered it unable to be absorbed by the root, while in the dilute solution, nitrate was easily absorbed; thus the absorption of nitrogen was different in the two experiments, hence the different growth resulted.

Experiment 4

NH_4NO_3=0.00122 N.

The results of this experiment are summarized in Table 4. It is interesting to see that an almost equal amount of ammonia and nitrate was absorbed by the roots from the very beginning of the experiment. Though in the middle stage of the experiment, the difference between ammonia and nitrate absorbed became a little greater, the absorption ratio NH_3/NO_3 was never beyond 2.

Owing to the diluteness of the culture solution, the reaction of the medium changed gradually to the acid side, though nitrate was relatively better absorbed in this case than in the foregoing experiments. Ammonia was exhausted on the tenth day; after that time, only nitrate was absorbed and consequently the acidity of the solution was lessened.

The growth of seedlings in the solution was excellent, at least as good as that in Experiment 2 where the concentration of the solution was just ten times that of this experiment. Up to the tenth day, the dry weight of root of the seedlings grown in both solutions was about the same. From the tenth day on, the seedlings in the concentrated solution grew better in every respect than in the dilute solution. No doubt, lack of nitrogen in the dilute series was the cause of this inferior growth.

Experiment 5

NH_4NO_3=0.00012 N.

As the salt concentration of the culture solution was extremely dilute, almost the whole amount of nitrogen was absorbed by the roots 2 days after the beginning of the experiment. However, a trace of nitrate remained in the solution, since the micro-chemical test with diphenylamin showed a positive reaction. It is clear that even in the case of extremely dilute solution, the unequal absorption took place too. The pH-value of the solution was shifted from 5.6 to 5.2.

From the results of the experiments described above, it became clear that the unequal absorption of cation and anion of salt took place in both the concentrated and the dilute solutions when the seedlings were grown in them.

In the case of concentrated solutions such as those in Experiment 1 and 2, no nitric nitrogen was absorbed by the roots at all, and even in a solution of moderate concentration, the absorption of nitrate was very slow during the first half of the experiment. On the contrary, the root system took up nitrate very easily and rapidly in the dilute solutions. The ratio of absorption NH_3/NO_3 was great in the case of the concentrated solution, but was comparatively small in dilute ones. Text-fig. 1 shows graphically the relation between the absorption ratio and concentration of solution. Here the ordinate represents the absorption ratio, while the abscissa denotes the experimental duration in days. It is evident that the higher the concentration of the solution, the greater the absorption ratio.

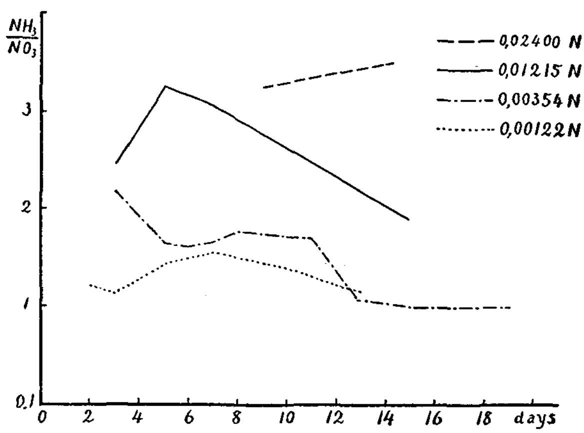


Fig. 1

The relation between the unequal absorption of ammonia and nitrate and the growth of the seedlings as well as the change of reaction in the solution is rather complex. In the case of concentrated solutions, the roots of seedlings absorbed ammonia only rapidly at first, causing the sudden increase of acidity in the solution. This high acidity, together with the great concentration of the solution, rendered the absorption of salt and the growth of roots impossible, and the seedlings fell to the ground. In the case of the dilute solution, however, the result is different. The young plants can utilize nitrate almost as well as ammonia. As a result of this, the reaction of the culture solution changed relatively slowly. This point is favourable to the growth of seedlings. But owing to the weak buffer action of such dilute solution, the reaction changes ultimately to pH 3.0. This high acidity is not extremely unfabourable to the growth of seedlings, because the absorption of nitrate in acid solution usually occurs without much hindrance in dilute solution, while it is very difficult or simply impossible in the case of concentrated solutions.

The increase of acidity in the culture solutions mainly depends upon the difference between the amounts of ammonia and nitrate which were absorbed by the roots; the extreme acidity always appeared in the solution where this difference was the greatest. But as the buffer power of these solutions was different from each other, difference of between NH_3 and NO_3 which caused the greatest change of pH of the solution, was also different. It should be noted, however, that in such complete nutrient solutions, the unequal absorption of cations and anions other than ammonia and nitrate must take part in the change of the reaction of the solution. The ultimate increase or decrease of acidity must depend on the difference between the sum of all cations and the sum of all anions which remained in the solutions. And indeed HAAS and REED (1926) actually found that in the water culture of Citrus seedlings the reaction of the solution changed to the more acid side when the sum of cations absorbed was greater than the sum of anions and vice versa. But it is a well-known fact that the absorption of nitrogen by plants is greater than that of any other salts. Therefore in such solutions as those used in these experiments, where the nitrogen content was far greater than the other salts, no doubt, the unequal absorption of ammonia and nitrate plays a predominant part in the change of reaction.

B. The concentration of ammonium nitrate only varied

The results of the foregoing experiments show clearly the influence of the concentration of the culture solution upon the unequal absorption of ammonia and nitrate. Another series of experiments was carried out in which only the concentration of ammonium nitrate was varied, while the concentration of other salts was kept constant. The composition of the solution was as follows:

No.	Stock solution		H_2O	Sum cc.	Concentration	pH
	A (cc.)	B (cc.)	cc.		of NH_4NO_3	
1	200	200	3600	4000	0.01192	4.8
2	”	100	3700	”	0.00593	”
3	”	70	3730	”	0.00420	4.7
4	”	40	3760	”	0.00242	4.8
5	”	20	3780	”	0.00121	4.7

Three seedlings were grown in each culture containing 400 cc. of nutrient solutions.

Experiment 6

NH_4NO_3=0.01192 N.

This solution was practically the same as that used in Experiment 2. As we have seen before, the absorption of nitrate was very slight at the commencement of the experiment. After the roots had absorbed ammonia to some extent, the absorption of nitrate began. The ratio of absorption NH_3/NO_3 was always greater than 2 except at the last harvest. As a matter of course, the acidity of the solution became greater and greater as the difference $[NH_4]-[NO_3]$ grew larger and larger. As the result of high acidity, the root system of the seedlings began to decay on the llth day and dry weight of the last harvest decreased as shown in Table 5.

Experiment 7

NH_4NO_3=0.00593 N.

The plant grown in this solution was not better than that in the 0.01192 N solution. This is a result which might be expected from the manner of change of reaction which was almost the same as in the foregoing experiment. The difference between ammonia and nitrate absorbed also resembles that in Experiment 6. The ratio NH_3/NO_3 was comparatively small, especially two days after the beginning of the experiment; that is to say in the former case it was 43, while in this case it was 4.86.

The result of this experiment (Table 6) shows that the dilution of one salt in a complete solution also has influence on the unequal absorption of cations and anion of that salt.

Experiment 8

NH_4NO_3=0.00420 N.

The analytical results are shown in Table 7. It is surprising to see that from the very beginning of the experiment, nitrate was sbsorbed more rapidly than ammonia, a fact which has never been seen before. In the foregoing series of experiments, we have seen that when the concentration of the solution becomes dilute, the divergence between cations and anions absorbed becomes less. But the absorption ratio NH_3/NO_3 was always greater than unity, and $[NH_4^{\cdot}]-[NO_3']$ was always positive. In the case of the solution whose concentration of NH_4NO_3 was 0.00354 N, the ratio was never less than 1.5 till on the 12th day when the ammonia content was exhausted. Notwithstanding the comparatively higher concentration of NH_4NO_3 in the present case, the absorption of nitrate was greater than that of ammonia from the commencement till the seventh day of the experiment. The absorption ratio NH_3/NO_3 was always less than unity; on the fourth day it was 0.80. From the seventh day on, the absorption of ammonia predominated and the ratio became 1.12 on the seventh day and 1.29 at the end of the experiment.

In spite of the superior absorption of nitrate, the reaction of the solution changed gradually to the acid side. Here the difference between ammonia and nitrate had no great influence on the increase or decrease of acidity of solution. On the first day when the difference $[NH_4^{\cdot}]-[NO_3']=-2$, the pH-value shifted from 4.7 to 3.7, and on the fourth day the difference was −42, while the pH-value of the solution became 3.0. However, when the absorption of ammonia became dominant and the difference between ammonia and nitrate absorbed became 30, 39 and 78, the pH-values of the solutions also changed to 2.9, 2.9 and 2.8 respectively a phenomenon which shows that the difference of ammonia and nitrate absorption has more or less concern in the change of reaction of the solution.

The growth of seedlings in this case resembled in general that of the foregoing case.

Experiment 9

$NH_4NO_3=0.00242$ N.

In this case, the superior absorption of nitrate to that of ammonia is much more obvious. On the second day of the experiment the nitric nitrogen absorbed was about 30 mg. per one gram of dry root, while only about 25 mg. of ammoniacal nitrogen were taken up by the same amount of dry root, the ratio NH_3/NO_3 was 0.88. This superior absorption of nitrate lasted for 5 days. From the 6th day on, the absorption of ammonia predominated and the ratio NH_3/NO_3 approached unity (Table 8).

As in the former case, the pH-value of the solution shifted from 4.8 to 3.5, 3.4, 3.3 and 3.2 as the experiment went on, though the differences

$[NH_4^{\cdot}]-[NO_3']$ were respectively -8, -28, -32 and -40, as if the increase of acidity was inversely proportional to this difference. When the absorption of ammonia became dominant, the acidity of the solution increased a little more. But as the nitrogen content of the solution was nearly exhausted on the ninth day, the pH-value remained constant till the end of the experiment.

The growth of seedlings was tolerably good, especialy the root system. It is a noteworthy fact that the growth of the root became better when the nitrogen content of the solution became less and that the root grew very well when practically all the nitrogen source was exhausted. No doubt, the existence of other nutrients in the solution was the cause of this good growth.

Experiment 10

This experiment was a repetition of Experiment 9. The results are shown in Table 9.

The results of this experiment resembled those of the foregoing in every respect. In the first 24 hours, the amount of ammonia absorbed was only two thirds that of nitrate; the absorption ratio NH_3/NO_3 was 0.68. This ratio drew near to unity as the experiment went on. The acidity of the solution increased at first, but from the fourth day on, it remained constant as previously seen in Experiment 9.

Experiment 11

$NH_4NO_3=0.00121$ N.

Owing to the diluteness of the solution, the absorption ratio NH_3/NO_3 in this case was almost equal to unity, except at the first harvest where it was 0.82 (Table 10).

The acidity of the solution increased at the commencement of the experiment but became constant as soon as the source of nitrogen was exhausted.

As the root system of the seedlings continued its growth in spite of the exhaustion of the nitrogen source, the amount of nitrogen absorbed per one gram of dry root became less and less after the fourth day.

Experiment 12

This experiment was a repetition of Experiment 11. The culture solution used was different in composition, though the concentration of ammonium nitrate was practically the same. In order to avoid the trouble of removing chlorine with silver sulphate in the determination of nitrate, a culture solution free from chlorine was prepared. Its composition was as follows:

Stock solution A′

KH_2PO_4	2.5 gm.
$MgSO_4 \cdot 7\ H_2O$	″ ″
Distill. water	500 cc.

Stock solution B′

NH_4NO_3	1.82 gm.
0.1 N $(NH_4)_2SO_4$	17.5 cc.
0.069 N $Ca(NO_3)_2$	25.3 ″

make up with distilled water to 1000 cc.

A′	50	cc.
B′	50	cc.
H_2O	900	cc.
Sum	1000	cc.

Three seedlings were grown in each culture containing 350 cc. of the culture solution. The composition of this solution was different from that of the preceding case in that it lacked Cl which was replaced by an equimolar amount of SO_4. The experiment was carried out in duplicate. From Table 11, it is clear that the results were fairly in harmony with those of the foregoing experiment.

From these results described in Experiments 6–12, it may be concluded that when other nutrient elements were kept constant and only the concentration of the nitrogen source varied, the manner of unequal absorption was somewhat different from that when the concentration of the whole solution was different. When the concentration of ammonium nitrate was diluted to a certain degree, superior absorption of nitrate always occurred. Text-fig. 2 shows clearly these relations. The absorption of ammonia was predominant when the concentration of ammonium nitrate only was diluted to 0.00593 N. But in a solution whose concentration of ammonium nitrate was only a little more dilute, say 0.00420 N., the amount of nitrate absorbed surpassed that of ammonia, especially at the commencement of the experiment. This superior absorption of nitrate became greater when the concentration of ammonium nitrate became more dilute.

It is interesting to see that in the solution of the same concentration of ammonium nitrate, for example 0.00122 N, the manner of absorption of ammonia and nitrate in a solution, in which the solutes all were diluted, was quite different from that in a solution whose ammonium nitrate only was diluted. In the former case, though the divergence of unequal absorption of ammonia and nitrate became less by dilution, the amount of ammonia absorbed was always greater than that of nitrate. But in the latter case, the relation is the reverse; from the very beginning of the experiment, more nitrate was absorbed by the roots. The difference of these two solutions was that in the one the concentration of the whole contents was diluted, while in the other only ammonium nitrate was diluted, but the concentration of other ions besides ammonium and nitrate was far greater than that of the source of N. Therefore, it is not without reason to ascribe the superior absorption of nitrate to this superfluous existence of other ions. The concentration of the source of nitrogen in usual culture solutions as well as in our solutions used in the first series of experiments, is much greater than that of other salts. The influence of these salts other than the source of nitrogen upon the absorption of nitrogen may be very little, if any, and can be neglected. But if the concentration of the nitrogen source only were diluted to such an extent that it was very small compared with that of other salts, the ions of other salts may exert a powerful influence upon the unequal absorption of the cations and anions of the nitrogen source. That this is the case will be apparent from the results of the following experiments. On the other side, from the manner of change of the reaction in the solutions, it may be concluded that a considerable amount of cations was absorbed by the plants. The intake of the cations in excess may be accompanied by the absorption of anions, and nitrate is indeed one of the anions that can be rapidly ab-

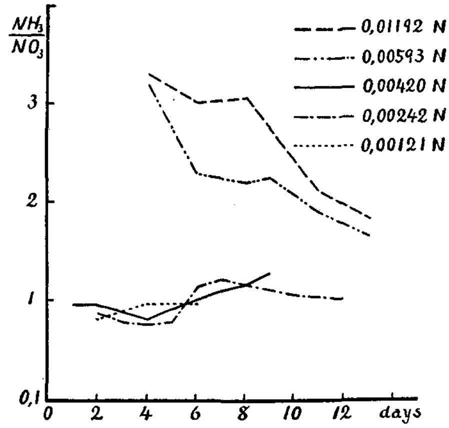


Fig. 2

sorbed by the plants.

The other notable point is that in spite of the superior absorption of nitrate to ammonia, the reaction of the solution always changed to the acid side. In this case, the difference of velocity of absorption between ammonia and nitrate seems to be indifferent to the pH-value. This might be expected from the influence of salts other than the source of nitrogen when they exist in overwhelming quantities. In such solutions the absorption of the salts other than ammonium nitrate must be far greater than the absorption of the later salt, and therefore the unequal absorption of cations and anions of the salts besides ammonium nitrate must play a considerable part in the change of reaction of the solution. Of course, difference between ammonia and nitrate absorbed also took part in this change, especially in the later part of the experiment. Compare the pH-change in Tables 7 and 8 it will be seen that in the solution which contained greater amounts of ammonium nitrate, the pH-value changed to 2.7, while in the solutions containing smaller amounts of ammonium nitrate, it changed to pH 3.0 and remained constant as soon as the source of nitrogen of the solution was exhausted. Therefore the difference between ammonia and nitrate absorbed as showed in Table 7 had at least some concern with the high acidity of the soltion.

Though in the solution in which the concentration of ammonium nitrate was low the absorption of nitrate was superior to that of ammonia, this superiority did not last long and from the middle of the experiment the absorption of ammonia predominated again. This phenomenon can be explained in the following way. The absorption of salts by plants depends chiefly on their requirement of the salt as nutrients. Between ammonia and nitrate, the former seems to be better assimilated by plants than the latter, since nitrate is believed to be assimilated after reduction to ammonia in the process of protein synthesis. Consequently for a moment the absorption of nitrate predominated over that of ammonia under the influence of other salts, but the accumulation of nitrate in the tissue and the greater utilisation of ammonia make the absorption of ammonia superior to that of nitrate.

The growth of seedlings in these experiments was equally good, except that the root systems grown in the solutions containing a greater quantity of ammonium nitrate looked unsound at the end of the experiment, while those in the solutions containing a smaller quantity of ammonium nitrate grew very well throughout the experiment.

C. The concentration of the salts other than the nitrogen source varied

The results of the foregoing series of experiments show that the unequal absorption of cations and anions of a salt is affected by the presence of other ions when they exist in excess. Thus, other salts being constant, the dilution of ammonium nitrate of a solution may cause the superior absorption of nitrate over ammonia. In oder to ascertain this influence of concentration of other salts besides ammonium nitrate, a series of experiments was carried out in which the concentration of ammonium nitrate was kept constant, but the concentration of other salts was changed. The composition of the solutions was as follows:

No.	Stock solution		Distill. water (cc.)	Sum cc.	Conc. of salts besides NH_4NO_3	pH
	A (cc.)	B (cc.)				
1	100	15	885	1000	2 Knop	4.6
2	50	”	935	”	Knop	”
3	45	”	940	”	$\frac{5}{6}$ Knop	”
4	25	”	960	”	$\frac{1}{2}$ Knop	4.8

Three seedlings were grown in each culture containing 350 cc. of nutrient solutions in which the concentration of ammonium nitrate was 0.00354 N. Under normal conditions, the absorption of ammonia and nitrate in such a solution is not very divergent (see Table 3).

Experiment 13

Concentration of salts twice as great as that contained in Knop solution

The contents of salts other than ammonium nitrate in this solution were twice as great as usual. Their influence was apparent. From Table 12, it will be seen that the absorption of nitrate was predominant over ammonia; the ratio of absorption NH_3/NO_3 up to the 8th day was less than one. As has been noted before, in a usual solution which contains 0.00354 N of ammonium nitrate, the amount of ammonia absorbed always surpasses that of nitrate, though the divergence of the absorption is not very great between the cations and anions.

Though the difference $[NH_4^{\cdot}]-[NO_3']$ was negative, the reaction of the solution became more acid.

The growth of the seedlings was tolerably good at first, but became a little weaker at the end of the experiment.

Experiment 14

Concentration of salts equal to that in KNOP solution

The amount of salts other than ammonium nitrate in this solution was equal to that of the usual one, but as the concentration of ammonium nitrate was very dilute, it exerted more or less influence on the unequal absorption of cation and anion. The results of this experiment (Table 13) show that the ratio of absorption NH_3/NO_3 was near to unity, sometimes the absorption of nitrate predominated, while sometimes much more ammonia was absorbed. Perhaps due to the presence of this amount of salts other than ammonium nitrate, the velocity of the absorption of ammonia and nitrate into the plant tissue was almost equal to each other.

As in the former case, the difference of absorption $[NH_4^{\cdot}]-[NO_3']$ had no appreciable effect on the change of reaction.

The growth of seedlings, in general, also resembled that of the former case.

Experiment 15

Concentration of salts other than NH_4NO_3 equal to $\frac{5}{6}$ that in KNOP solution

In this solution, ammonia was always better absorbed than nitrate, though the divergency of unequal absorption was very small. The ratio of absorption NH_3/NO_3 was almost equal to unity, the maximum was 1.3. Here we see also that the presence of salts other than ammonium nitrate had at least some effects on the unequal absorption of ammonia and nitrate (Table 14).

In this case, the difference between ammonia and nitrate absorbed had more or less concern with the change of acidity of the solution. The increase of acidity was directly proportional to this difference. The maximum increase of acidity occurred on the 6th day when the difference was 0.00074 N. From that day on, the acidity decreased with the diminution of difference.

The growth of seedlings was exceedingly good. At the end of the experiment almost all the ammonium nitrate had been absorbed by the plants.

Experiment 16

Concentration of salts other than NH_4NO_3 equal to one half that in KNOP solution

The results of this experiment are shown in Table 15. It will be seen that in the commencement of the experiment the superior absorption of ammonia to nitrate was very remarkable. But in the middle part of the experiment the divergence of this unequal absorption was much lessened. On the whole, the amount of nitrate absorbed was but little greater than that shown in Table 3.

The presence of the salts other than ammonium nitrate also played a part in the change of reaction of the solution. The acidity of the solution increased gradually from pH 4.8 to 2.9 in spite of the difference between ammonia and nitrate absorbed. The growth of seedlings in this case was less favourable in comparison with that of the foregoing cases.

The results described above exhibit clearly the influence of salts other than ammonium nitrate itself upon the velocity of absorption of ammonia and nitrate when they exist in a great quantity. The absorption of ammonia by the root system in a solution containing 0.00354 N ammonium nitrate and of other salts whose concentration was just ½ of KNOP solution was always superior to that of nitrate. But if the concentration of salts other than ammonium nitrate was increased as much as twice that of KNOP solution the relation was just the reverse: the absorption of nitrate always predominated. When the amount of salts in the same solution was reduced to a concentration just equal to that of KNOP solution the ratio of absorption of ammonia and nitrate was near unity; if reduced to a concentration less than that of KNOP solution, the absorption of ammonia predominated again. Thus, when the concentration of ammonium nitrate of a solution is constant, the rate of absorption of nitrate can be accelerated or reduced by increasing or decreasing of other nutrient elements. The amount of salts required for accelerating the absorption of nitrate beyond that of ammonia seems to be dependent upon the concentration of the ammonium nitrate in the solution. In the present case, the concentration of ammonium nitrate was 0.00354 N, and the amount of salts required to obtain the superior absorption of nitrate was greater than that contained in KNOP solution.

As to the nature of this influence of salts, two probabilities can be considered:

1. As denoted before, from the manner of change of acidity it may reasonably be concluded that a great quantity of cations besides ammonia was absorbed by the roots. Perhaps, the great part of nitrate was attracted by the penetration of cations into the tissue.

2. The accumulation of cations other than ammonia in the root tissue retards the velocity of absorption of ammonia.

We shall discuss these points later.

In such solutions which contained a much greater quantity of other salts in comparison with ammonium nitrate, the change of reaction was not wholly dependent upon difference between the ammonia and nitrate absorbed. When the amount of salts was great, the increase of acidity had no relation whatever to this difference, as the pH-value changed from 4.6 to 2.9 in spite of the superior absorption of nitrate. In a solution of lower concentration of other salts, the difference between ammonia and nitrate absorbed seemed to have more or less concern with the reaction change; but in a solution of which salt content is just one half of that in KNOP solution, the effect of the difference between ammonia and nitrate absorbed upon the reaction was also unremarkable. To be brief, in such cases, the unequal absorption of cations and anions other than ammonia and nitrate plays a great rôle in the increasing or decreasing of acidity of the culture solution.

The growth of seedlings was the best of all in a solution containing salts five sixths of that of KNOP solution. The growth in the other solutions resembled each other. The chief cause of this was perhaps due to the difference of acidity of the solution, that is: in other solutions, the acidity increased steadily to as great as pH 2.9 or 2.8, while in this solution, though it became pH 2.9 on the 6th day, the acidity decreased again, owing to the exhaustion of ammonia.

D. Single NH_4NO_3 solution of varied concentrations

The data of experiments described thus far exhibit clearly the influence of concentration of the culture solution and the effect of salts other than the source of nitrogen in the solution upon the unequal absorption of ammonia and nitrate. The nutrient solutions used in these experiments consist of many elements which are thought to be necessary for the normal growth of plants. On the other hand, however, the presence of so many kinds of salt in one solution leads to antagonistic and other effects upon the absorption of a particular constituent. And indeed the results described in parts B and C show that this was the case. Therefore, it is desirable to study the absorption of ammonia and nitrate in a single solution of ammonium nitrate of different concentrations.

The unfavourable effects of a single salt solution on the physiological function of plants have been reported by many authors. A solution for a physiological study, therefore, must always contain more than one salt, that is: it must be a balanced solution. A solution of a single ammonium salt may be toxic to the growth of seedlings. But when the duration of the experiment is short, and the concentration of the solution low, seedlings may grow in it without any serious injury. Accordingly the following four experiments were carried out with a single ammonium nitrate solution, the concentration of which was varied from 0.00483 N to 0.0063 N. The duration of the experiment was 12 days except in the case of the most dilute solution which lasted only one week. Three seedlings were grown in each culturs containing 350 cc. of the solution.

Experiment 17

NH_4NO_3=0.00483 N.

The seedlings grew in this solution quite well without any injury appearing in the shoot or in the root, especially during the first part of the experiment. Owing to the lack of iron in the solution, chlorosis took place in the shoot on the seventh day.

The results of this experiment are summarized in Table 16. In general, the data closely resemble those of the complete solution. The amount of nitrate absorbed at the beginning of the experiment was very small, only one sixteenth of that of ammonia. The divergence of this unequal absorption became less from the third day on, yet the ratio of absorption NH_3/NO_3 was always greater than 1.5.

From the seventh day on the absorption of ammonium nitrate was retarded partly by the increase of acidity in the solution and partly by the chlorosis of leaves. 70 mg. of ammoniacal nitrogen and 35 mg. of nitric nitrogen were taken in by the roots in the first week, but only 6 mg. of ammoniacal and 10 mg. of nitric nitrogen were absorbed after the 7th day.

The reaction of the solution changed rapidly to the acid side. This change of the pH-value gradually became slower when the absorption was retarded. In general, the increase of acidity was proportional to the difference between the ammonia and nitrate absorbed; the greater the difference, the more acid the solution. Though the difference between ammonia and nitrate absorbed was by no means great, the pH-value became 3.8 on the first day and 2.8 at the end of a week. Perhaps the lack of any buffer in the solution may be responsible for this rapid change of the reaction.

Experiment 18

$NH_4NO_3 = 0.00354$ N.

Table 17 shows the results of the experiment. In this case, nitrate was comparatively better absorbed, 5.61 mg. of nitric nitrogen being taken up per one gm. of dry weight of root against 16.5 mg. of ammoniacal nitrogen. The ratio of absorption NH_3/NO_3 was about 3 at the beginning and usually less than 1.5 during the whole duration of the experiment.

The reaction of the solution was as rapidly changed as in the former case. Here, too, the difference between the absorption of ammonia and nitrate played a great rôle in the increase of acidity. The high acidity of the solution also hindered the absorption in the later half of the experiment.

Experiment 19

$NH_4NO_3 = 0.00246$ N.

The growth of seedlings in this solution was just as good as in the former two solutions. Chlorosis appeared in the leaves on the seventh day of the experiment.

Table 18 exhibits better absorption of nitrate in this solution than in the former experiments with solutions of greater concentrations. The ratio of absorption NH_3/NO_3 was only 1.75 at the commencement and became less in the later part of the experiment.

As the difference between ammonia and nitrate absorbed was comparatively small, the pH-value of the solution changed gradually to the acid side. The degree of increase of acidity was proportional to the difference of the absorption.

Experiment 20

NH_4NO_3=0.00063 N.

The results of this experiment are shown in Table 19.

Owing to the diluteness of the solution, all of the salts were absorbed on the seventh day of the experiment; therefore the experiment was discontinued. In fact, the ammonia content of the solution was already exhausted on the third day, though a trace of nitrate remained in the solution till the lapse of one week.

Practically no divergence of absorption between ammonia and nitrate was found in this experiment, almost an equal amount of them was taken in by the plant from the very beginning. The ratio of absorption NH_3/NO_3 was about 1.2 on the first day and became almost unity in the later part of the experiment.

The reaction of the solution was changed from pH 5.4 to pH 3.9 during the first 24 hours, but this acidity was soon lowered as the difference between the amounts of ammonia and nitrate absorbed became less. At the end of the experiment, when all the salts were exhausted, the pH-value was 4.8. It was more acid than at the beginning. This acidity was due to the production of carbon dioxide in the solution by the respiration of the root system. By driving out the carbon dioxide the pH-value of the solution became 7.8; this is the pH-value of the distilled water used in this experiment when it is free from carbon dioxide.

The result of the absorption of ammonia and nitrate in the single ammonium nitrate solutions were practically the same as those in the complete solution. We have seen from the date above described that in single ammonium nitrate solutions the divergence of unequal absorption became less as the concentration of the solution became dilute. In the concentrated solutions, little or no nitrate was absorbed at the commencement of the experiment, while in the dilute solutions almost an equal amount of nitrate and ammonia was absorbed at the very beginning. The ratio of absorption NH_3/NO_3 was greater in the concentrated solutions than that in the dilute ones (Text-fig. 3.). We have seen, too, that this superior absorption of ammonia in the concentrated solutions always caused

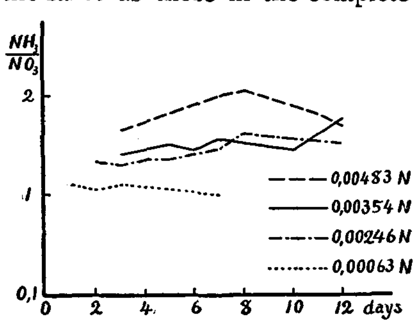


Fig. 3

a sudden and continuous increase of acidity in the solution which usually hindered the growth and absorption.

As in the case of complete solutions, the change of reaction of the solution of ammonium nitrate was proportional to the difference between ammonia and nitrate absorbed. But in this case, the amount of nitric acid which was left in the solution and was responsible for shifting the initial pH of the solution to a certain pH-value, was of great variation. For example, the differences of ammonia and nitric acid which caused a shift in the acidity are shown as follows:

pH 5.4 to pH 3.8	0.00033—0.00044 N,
,, ,, ,, ,, 3.0	0.00033—0.00061 N,
,, ,, ,, ,, 2.8	0.00082—0.00120 N.

What is the cause of this variation? First of all, the excretion of acid substance from the root tissue may be considered. The excretion of substances from plant cells into the culture solutions has been reported by many authors. REDFERN (1922) found that the seedlings of *Pisum sativum* and *Zea Mays* absorbed calcium ions in excess in the calcium chloride solutions, but the reaction remained almost neutral, that of the external solution showed the existence of Mg and K ions which had diffused out of the root. STOCKLASA, SEBOR, TYMICH and CWACHA (1922) concluded that the absorption of alminium and ferric ions by roots of *Eriophorum vaginatum*, *Phragmites communis* and *Carex riparia* in water culture is accompanied by the excretion of Ca, Mg and Na ions. STILES (1924 b) found that the storage tissue of many plants absorbed the ions unequally from single solutions of ammonium chloride, ammonium phosphate, ammonium sulphate, potassium chloride, sodium chloride and sodium sulphate, but the solutions remained neutral on account of the excretion of Cl, K, Ca and a trace of Mg. HOAGLAND, HIBBARD, and DAVIS (1926) working with *Nitella* cell, found the excretion of Cl ion from the cell accompanied by the accumulation of Br ion. HAAS and REED (1926) observed the excretion of K from Citrus and walnut seedlings in complete solution without potassium salts. TUEWA (1926) found the excrction of K and Ca from the root system of wheat in distilled water and buffer solutions. In the latter case, the excretion of Ca and K always took place in the solution of acid reaction. She also found that Ca was usually excreted by seedlings older than 5 weeks, but the excretion of K was observed in the case of younger seedliegs. OSSIPOWA and JUFEREWA (1926) observed the excretion of SO_4 and PO_4 ions from the root of seedlings of wheat and maize. MINANA (1927) recognized the excretion of organic acid by cultivated plants in distilled water within 24 hours. This fact was also ascertained by SABININ and MINANA (1928). Recently NIKLEWSKI, KRAUSE and LEMANCZYK (1928) observed the excretion of Ca, Mg, HCO_3 and HPO_4 ions within 18 hours from barley seedlings. As the presence of other ions has very great connection with the absorption, the writer has carried out experiments to test the fact of excretion. Seedlings of *Zea Mays* were grown in single ammonium nitrate solution after the preliminary culture for 48 hours. Tests for K, Ca, Mg, Cl, PO_4, and SO_4 ions in the external solution were made qualitatively(1) in addition to the quantitative analyses of ammonia and nitrate. The number of seedlings was varied from 3 to 6, and all the cultures were triplicated. The tests of the excretion of these ions resulted all in negative. Next, the same tests were carried out with a series of solutions containing a mixture of sodium phosphates and ammonium nitrate in which seedlings had grown for 48 hours. The pH-value of the solutions was varied from 3.0 to 8.5. Here also no excretion was observed. But when disks of beet turnip-root were immersed in ammonium nitrate solutions for 24 hours, excretion of K, Ca, Mg, NH_4, Cl and PO_4 was always ascertained. The experiment with turnip was repeated with the similar result. Therefore it must be remembered that the absorption of a storage tissue such as turnip is wholly different from that of an absorbing organ. Under our conditions, little or no substance except carbon dioxide which is a product of respiration of the root system, and probably phosphatides might be excreted from the root system.

Then, it comes to be concluded that carbon dioxide played a part in shifting the reaction of single salt solutions. As has been noted before, the final pH of a solution, the salt content of which has been absorbed by the root system, was 4.8, but became 7.8 when the carbon dioxide was driven out.

Now the question arises whether the carbon dioxide has anything to do with the change of reaction in complete solutions. Since these solutions contained buffer to some extent, carbon dioxide must have little or no effect on the change of the reaction. In an early paper (1928), the writer pointed out the fact that the degree of change of reaction was equal between a solution which was aërated with CO_2-free air and one that was not aërated.

(1) The method of the test was as followos: for K, cobalt nitrite method; for Ca, oxalate method; for Mg, NH_4-molybdate method; for Cl, with $AgNO_3$; for PO_4, colorimetrical method of Denige-Atkins; and for SO_4 with $BaCl_2$.

The amount of nitrogen absorbed was greater in the first part than in the later part of the experiment. A glance at Tables 16–19 will make clear the fact that increase of acidity always caused the retardation of the absorption in the later stage. When the acidity of the solution became greater than pH 3.0, the amount of absorption per one gram of dry root increased very little or not at all.

* * *
* *

The results of these experiments from 1 to 20 recorded above show clearly the important influence of the concentration of solution, both balanced and unbalanced, upon the unequal absorption of ammonia and nitrate from ammonium nitrate by the seedlings of *Zea Mays*. In the solutions of high concentration, the absorption of ammonia always surpassed that of nitrate, but the divergence of this unequal sbsorption between ammonia and nitrate became less when the concentration of the solution was diluted. When the concentration of salts other than ammonium nitrate was kept constant, and that of ammonium nitrate only was diluted to a certain degree, the amount of nitrate absorbed was greater than that of ammonia. Similar results were obtained when the concentration of the salts other than ammonium nitrate was increased to some extent while the concentration of ammonium nitrate was kept constant.

The influence of concentration of solution upon the unequal absorption has been studied by REDFERN (1922), STILES (1924 b), PRIANISCHNIKOW (1927), DAVIDSON (1927) and NIKLEWSKI, KRAUSE and LEMANCZYK (1928). REDFERN carried out a series of experiments on the absorption of calcium and chlorine ions from the solution of calcium chloride by the seedlings of *Zea Mays* and *Pisum sativum* and found that the more dilute the solution the less the divergence between the unequal absorption of the two ions. The concentration of solutions in REDFERN's experiment was from 0.1 N to 0.0001 N.

STILES investigated the unequal absorption by the storage tissue of carrots from solutions of ammonium chloride and sodium chloride with concentration varied from 0.1 N to 0.001 N. With both salts the absorption of two ions approach nearer equality the more dilute the solution.

Similar results have been secured by PRIANISCHNIKOW (1927) in the absorption of ammonia and nitrate by the seedlings of oats and peas from the solution of ammonium nitrate. He found that pea plants, both normal and etiolated young plants, absorbed regularly more ammonia than nitrate in the solutions with the concentration varied from 0.1 N to 0.0001 N, but in a dilute solution of 0.00001 N the amounts of nitrogen were exhausted within the duration of the experiment (4-6 hours), therefore no difference between the absorption of ammonia and nitrate could be observed. Though PRIANISCHNIKOW himself did not give any attention to the rate of absorption between ammonia and nitrate in these concentrations, the data of his work show clearly the fact that the ratio of absorption NH_3/NO_3 was proportional to the concentration; the greater the concentration, the larger the ratio of absorption.

On the other hand, DAVIDSON (1927), working on the sbsorption of phosphorus and potassium by wheat seedlings, came to the conclusion that the general character of the relative absorption was not affected by the concentration of the solution, provided the difference in initial reactions in the solutions of the lower concentration was maintained by daily renewal. In our case of single ammonium nitrate solutions the initial pH-values of the solutions of different concentrations were all equal, but the influence of concentration upon the unequal absorption was very apparent.

As to the cause of this influence of concentration of the culture solution upon the unequal absorption, surely the velocity of penetration of the cation and anion into a cell plays more or less part in it. It is a well known fact that ammonia penetrates living cells with great readiness. Nitrate, on the other hand, enters with less speed. As might be expected, this difference of velocity of penetration between ammonia and nitrate hecomes greater with the increase of concentration of the solution and smaller with the decrease of concentration. In this place, that this velocity of penetration of ammonia and nitrate into living cells has nothing to do with the mobility of ions, is apparent from the fact that the mobility of ammonium and nitrate ion is practically equal.

NH_4=64.2
NO_3=60.8 (After NERNST, 1911, p. 366)

But if the velocity of penetration of ions only was responsible for the influence of concentration, the absolute amount of nitrate absorbed must be increased with the increase of concentration. That this is not the case is evident from the data recorded above. We have seen from Tables 1-4 and 16-19, that the absolute amount of nitrate absorbed in more dilute solutionions was either equal to or even greater than that in the solutions of greater concentrations. Recently, NIKLEWSKI, KRAUSE and

LEMANCZYK (1928) observed the influence of concentration on the absorption of nitrate from the solutions of calcium nitrate by the roots of barley seedlings. When the roots absorbed more nitrate than calcium ions in every concentration, the percentage of nitrate absorbed was decreased with the increase of concentration. They considered that the influence of concentration depends upon the change of the electrical charge of the cell colloid. It is really difficult to understand how this change of electrical charge in cell colloid takes place. Perhaps, NIKLEWSKI, KRAUSE and LEMANCZYK laid too great a stress on the change of electrical charge of plasma membrane in explanining the mechanism of absorption of electrolyte. Certainly such change plays a considerable rôle in the absorption of ions, but, as has been pointed out by WL. S. BUTKEWITSCH and W. W. BUTKEWITSCH (1929), it is not the only influence on the complex phenomenon of absorption. Indeed the experimental data hitherto described shows that the rate of absorption of ammonia and nitrate was changed by the concentration of the complete solution and of other salts besides ammonium nitrate which apparently has nothing to do with the electrical charge of plasma membrane. The works of SCHULOW (1909) and PRIANISCHNIKOW (1927) also show the effect of the age of seedlings of *Zea Mays* upon the unequal absorption of ammonia and nitrate from a solution of ammonium nitrate; older seedlings absorbed more nitrate than ammonia. This phenomenon can also be ascribed to the change which takes place in the plant body with its age.

The works of NATHANSOHN (1904 a, b), MEUER (1909), PANTANELLI (1915), STILES and KIDD (1919), REDFERN (1922 a, b), STILES (1924 b) and NIKLEWSKI, KRAUSE and LEMANCZYK (1928) all indicate that the rate of absorption increased with the increase of concentration. As far as the data of the present work shows, this is not the case with the range of concentrations used. No notable difference can be found in the total amount of nitrogen absorbed per one gram of dry weight of roots among various concentrations of both balanced and single solutions.

In the more concentrated solutions, the acidity became suddenly great as a result of great divergence of unequal absorption of ammonia and nitrate. This great acidity often caused an unfavourable growth of the seedlings. On the other hand, because the divergence of unequal absorption in dilute solution was very small, the reaction of these solutions changed gradually to the acid side and gave better results on the growth of seedlings. Though the acidity in the dilute solutions ultimately became very great, it would be decreased as soon as the amount of ammonia became exhausted.

Thus the decrease and increace of acidity were proportional to the difference between amounts of ammonia and nitrate absorbed; the greater the difference, the more acid the solution. But when the content of the salts other than ammonium nitrate was far greater than that of ammonium nitrate, the reaction changed to the acid side in spite of the superior absorption of nitrate. In this case, the reaction of change was controlled by the unequal absorption of cations and anions other than ammonia and nitrate. In short, the data of the exprериments recorded above all show the dependence of the development of acidity in the solution upon the unequal sbsorption, since there was no trace of diffusion of cations or anions out of plant roots great enough to be detected by chemical tests.

The unequal absorption, the development of acidity in the solution as the results of this unequal absorption, and their effect upon the growth of the seedlings, all these phenomena are affected by the concentration of the culture solution and by the salts other than the nitrogen source. Therefore in consideration of the relation between the growth of plants and concentration of culture solutions the fact of unequal absorption must be taken into account besides the osmotic relations. Recent investigations have shown that the simple osmotic theory is inadequate for the explanation of the mechanism of absorption in cells (STILES, 1924 b).

The absorption of ammonia and nitrate in relation to hydrogen ion concentration of the culture solution

In his earlier works, the writer has ascertained the fact that the inferiority of ammonium salts to nitrate as the source of nitrogen consisted in the physiological acidity developed during the culture period through the unequal absorption of ammonia and nitrate by the culture plants. By using a combination of ammonium salts such as ammonium sulphate and ammonium bicarbonate instead of a single one or by addition of buffer to the solution, the writer succeeded in the improvement of growth of culture plants to some extent. These results suggest the probability of the unfavourable influence of high acidity of a solution on the absorption of ammonia and other nutrient elements besides its effect on the growth of plants. And indeed, the results recorded in the foregoing part throw more or less light on the problem in question; we have seen that the absorption of both ammonia and nitrate was hindered whenever the acidity of the solution became greater than pH 3.0.

Since the introduction of the method for accurate determination of hydrogen ion concentration in our research field, a host of authors has applied this method for biological investigations. Among them, the literature on the relation between the reaction of culture media (including soil reaction) and the growth of the culture plants has been accumulated so extensively that it led ARRHENIUS and MEVIUS to write excellent monographs (ARRHENIUS, 1926; MEVIUS, 1927). But studies of the influence of hydrogen ion concentration on the absorption of salts are comparatively few. HOAGLAND (1919) found that in solutions with acid reaction (pH 5–5.5) the absorption of nitrate and phosphate ions was greater than from a neutral one (pH 6.8) of similar composition and the same total concentration. ARRHENIUS (1922) found that the intake of K, Ca, Mg, PO_4, NO_3 and SO_4 by seedlings of wheat and radish was strongly influenced by the hydrogen ion concentration. In the experiments with wheat, a greater amount of anions was absorbed in solution of pH 4. ARRHENIUS regarded this phenomenon as due to "the result of injurious action on the roots which favours penetration, and to a chemical combination, possibly due in part to the formation of protein salts" as "the reaction of the solution lies on the acid side of the isoelectric point of most proteins." The greater absorption of nitrate in an acid solution than in an alkaline one was also confirmed by THERON (1923) with higher plants and HOAGLAND and DAVIS (1923 a, b) with *Nitella*. KUSNETZOV (1925) found that the absorption of nitrate nitrogen by the mycelium of *Citromyces siderophilus* from acid solution was greater than an alkaline one (solution—$CaCO_3$), but the absorption of ammonia was far better in alkaline solution than in acid. NĚMEC and GRACANIN (1925) found that the absorption of phosphorus from potassium phosphate was greater in the acid solution than in the alkaline solution. According to PRIANISCHNIKOW (1926), the in crease of the acidity in a solution (by adding phosphoric acid) resulted in the greater absorption of nitrate than in the alkaline solution. DAVIDSON (1927) found that the absorption of phosphorus by wheat plants was greater in acid than in the alkaline solution; he ascribed the cause of this phenomenon also to the existence of the isoelectric point of the protoplasm of the wheat. SABININ and KOLOTOVA (1927) found that the absorption of K, Ca, and PO_4 by the seedlings of *Zea Mays* was affected by the reaction of the culture solution: in acid solutions (pH 4–5), anions were better absorbed, while in alkaline solutions (pH 7–8) a greater amount of cations was taken in. NIKLEWSKI, KRAUSE and LEMANCZYK (1928) recognized a similar phenomenon in the absorption of calcium and nitrate ions from a solution of calcium nitrate in barley seedlings. They stated: "so sind offenbar bei den oberhalb einer pH=ca. 4 liegenden Wasserstoffzahlen die Wurzelkolloide negativ geladen, wodurch eine stärkere Ca-Aufnahme bedingt wird. Umgekehrt dürfte in saureren Lösungen als pH=ca. 4 die NO_3-Aufnahme stärker sein, bedingt durch intensive H_3PO_4-Ausscheidung, die tatsächlich qualitativ beobachtet wurde. Demnach wäre eine Lösung mit einer pH=ca. 4 als isoelektrischer Punkt der Wurzelkolloide zu betrachten, ..."

MEVIUS and ENGEL (1929) found that the absorption of ammonia from solutions containing ammonium salts by seedlings of *Zea Mays* increased as the pH-value of the solution became greater and greater.

In their investigations of the influence of hydrogen ion concentration upon the absorption of salts by plants, some of the authors mentioned above have referred to the existence of the isoelectric point of protoplasm in plant tissue. When the rate of absorption is plotted against pH, a curve with two maxima is obtained and the minimum point between the two maxima is believed to be the isoelectric point of the protoplasm. As the problem in question is of fundamental importance in a theoretical point of view, it has been one of the main purposes of the present studies to make clear the relation of hydrogen ion concentration of the culture solution to the absorption of ammonia and nitrate. It is a matter of great interest to see whether the rate of absorption plotted against pH is a curve with two maxima or a wave-formed curve with more than two maxima as shown by SAKAMURA and LOO (1925) in the case of experiment with the hardening and softening of protplasm of *Spirogyra*.

In the sutudies of the influence of hydrogen ion concentration of culture solution on the absorption of salts, there are many difficulties in the technique of experimentation which had to be overcome. Firstly, one usually uses buffer solutions in the preparation of solutions of different pH-values in order to avoid any change of reaction during the culture period. The ionic concentration of such solutions is different with different ph-values. The effect of other ions on the absorption has been reported by LUNDEGÅRDH and MORAVEK (1924). HOAGLAND and his coworkers (1923, 1925, 1928) and also confirmed by the writer in the other experiments. Therefore the data of such experiments cannot be regarded as a result of the pure effect of hydrogen ion concentration. But it is absolutely impossible to prepare solutions of different pH without the co-existence of other ions. Accordingly, different kinds of buffer mixtures must be employed and in each kind of buffer mixture, solutions of a constant amount of cation or anion must be prepared and the results based on the data of the experiments with these buffer mixtures compared. If the results are generally in accordance with every experiment, those may be regarded as the results of the influence of hydrogen ion concentration. In fact, the writer used several kinds of buffer mixtures; namely, sodium phosphate mixtures, sodium oxalate mixtures, and complete solution + sodium phosphate mixtures. In each kind of buffer mixture, solutions of constant cations and anions were used in the experriment. By these methods, the effect of ions in the buffer mixture became evident and this was always taken into consideration in the interpretation of the results.

The next point which is difficult to control is the change of reaction of the culture solution. In order to obtain a normal growth of seedlings, the concentration of buffer mixture cannot be too strong. With moderate concentration of buffer mixture, the pH-value of solutions having weaker buffer power would change very rapidly within 24 hours, so that it is difficult for one to judge which pH-value is responsible for the effect in such a solution. Reducing the duration of experiment is indeed a method of avoiding this change of reaction. But by doing this the amount of absorption is so small and the difference between the absorbed amounts in different solutions is so insignificant that one cannot draw any accurate conclusion from such an experiment. The usual method of controlling reaction, by the additon of acid or alkali to the solutions from time to time, or the constant renewal of solution, is impossible in our case, since the former will result in the disturbance of the ionic relations in the solution while the latter is unapplicable in analytical works.

Thirdly the rate of absorption may be affected by the growth of the seedlings. Absorption is believed to be a different process from growth (see for example O. Arrhenius 1922). Assuming this statement to be true, the absorption of salt, however, cannot be independent of the salt requirement of the plant. Therefore, during the course of growth, salts which are better assimilated by the plant may be absorbed in greater quantity at the last, though they are only a little absorbed at first. Inversely, salts which are taken in in great quantity at first may be repelled by the plants if they are accumulated in the tissue without being utilized. Thus the form of the absorption curve when it is plotted against pH, may be changed to some extent when the duration of the experiment and other conditions such as temperature and light differ.

Lastly one who has experience in water culture knows the great divergence in the growth of seedlings between the triplicate or duplicate of the same culture containing buffer solution. If a similar relation exists in the absorption, practically no conclusion can be drawn from these experiments. Accordingly a preliminary experiment was conducted in which six seedlings were grown for 24 hours in the green house in 350 cc. of culture solutions containing 0.005 N ammonium nitrate and M/50 sodium phosphate mixture. Following are the results:

No.	NH_4 absorbed 0.00001 N	dry weight of root (gm)	N-absorbed per 1 gm. of dry root (mg)
1	48	0.1475	15.95
2	46	0.1415	15.95
3	51	0.1585	15.75
4	48	0.1480	15.80

From the above Table, it is evident that no divergence exists in the absorption in a buffer solution when it is expressed in the form of nitrogen absorbed per one gram of dry weight of root. The amount of nitrogen absorbed in 48 hours was rather small, if compared with the absorption in single solutions of ammonium nitrate (see page 7). This, perhaps, is due to the existence of other ions which were quite concentrated in the experiment above mentioned.

To conduct all experiments under exactly the same conditions is a thing which is practically impossible. Therefore the writer carried out about twenty experiments during 1927–1929 with several kinds of buffer mixtures and varied durations of time and observed points of agreement and difference between the results. Seedlings of *Zea Mays* exculsively were employed as the culture plant. They were brought up in the way described in the former part of this paper. After the preliminary culture, they were transferred to the buffer solution which contained 0.0035 N ammonium nitrate for a fixed time. This concentration was chosen, because the divergence between the absorption of ammonia and nitrate was relatively small and thus the increase of acidity in the solution due to unequal absorption of ammonia and nitrate may be lessened. The absorption of ammonia and nitrate was determined by analysis of the external solution. Unless otherwise noted, all the experiment were carried out in the green house.

A. Phosphate mixtures

Among several kinds of buffer mixtures, organic and inorganic, sodium phosphate, mixture, perhaps, is the most favourable one for biological studies Firstly it is not toxic to living cells as phthalate or acetate are. Secondly, both the cation and anion are poorly absorbed by the plant and its effect on the absorption is relatively insignificant as compared to K, Ca and other ions. Besides these good points, the sodium phosphate mixture possesses tolerable buffer power at comparatively low concentration and therefore is very suitable for our purpose. For these reasons, a sodium phosphate mixture was chiefly employed in the present study.

As stock solutions, M/5 H_3PO_4 (MERCK's guaranteed reagent), M/5 NaH_2PO_4 (KAHLBAUM pro analysi) and M/5 Na_2HPO_4 (KAHLBAUM, zur Enzymstudien) were prepared. A fixed amount of them was taken with burette and diluted to ten times to secure the required pH-value.

1. *Experiments with uniform amount of anions in buffer*

In this series of experiments, the amount of anions in each solution with different pH-values was equal. The amount of cations was consequently less in acid and greater in alkaline solutions.

Experiment 21

The first experiment was carried out for 15 hours. Three seedlings were grown in each culture containing 350 cc. of solution having the following compositions.

No.	pH	M/5 H_3PO_4 cc.	M/5 NaH_2PO_4 cc.	M/5 Na_2HPO_4 cc.	ca. 0.35 N NH_4NO_3 cc.	H_2O cc.	sum cc.
1	3.0	10	90	0	10	890	1000
2	4.2	0.5	99.5	0	"	"	"
3	4.5	0	100	0	"	"	"
4	5.0	"	99	1	"	"	"
5	5.5	"	95	5	"	"	"
6	6.0	"	85	15	"	"	"
7	6.5	"	74	26	"	"	"
8	7.0	"	49.5	50.5	"	"	"
9	7.5	"	25	75	"	"	"
10	8.0	"	6.5	93.5	"	"	"
11	8.5	"	0	100	"	"	"

The results of this experiment are shown in Table 20 and Text-fig. 4.

It will be seen from these data that within the time limits, no significant change of reaction took place in the solutions. This might be the result of small absorption by the plants. Though the difference of absorption among solutions of different pH was in general not very significant, the influence of hydrogen ion concentration of the solution upon the absorption of ammonia and nitrate, however, can be recognized. The absorption of ammonia was the least at pH 3.0. It became greater as the acidity of the solution became less and reached the first maximum at 4.5–4.4. Then it sank suddenly at pH 5.0. From this on, the absorption became greater again and reached the second maximum at pH 6.0. The absorption curve sank again at pH 6.5–6.4. A third maximum point of absorption was found at about pH 7.5 and a third minimum at pH 7.9. The absorption of nitrate was generally paralleled to that of ammonia. The maximum and minimum points of absorption of nitrate were coincident with those of ammonia except that the absorption of nitrate became less and less in the alkaline side where the pH-values of the solutions were greater than 7.5. This is in agreement with the findings of many authors who found the absorption of nitrate to be greater in an acid than in an alkaline solution. Thus within the pH-range of this experiment we have obtained three maxima and three minima of the absorption curve in the case of both ammonia and nitrate.

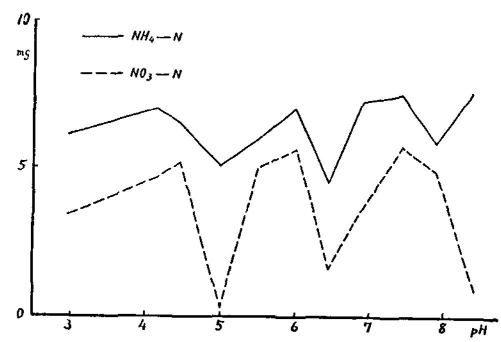


Fig. 4

On the whole, the absorption of ammonia was better in the alkaline than in the acid side. From the solutions whose pH-values were greater than 7, the amount of ammoniacal nitrogen absorbed per one gram of dry weight of the root was greater than 7 mg (with one exception at pH 7.9), while in the acid side it was always less than 7 mg. The favourable pH-range for the absorption of nitrate was between pH 5.5–6.0. When the pH became greater than 7.5, the amount of nitrate absorbed became less and less as has been noted.

Experiment 22

This experiment was only a repetition of the foregoing, but the duration of the experiment was a little longer than the first, being 24 hours instead of 15. The date have been summarized in Table 21 and illustrated in Text-fig. 5.

From Table 21, it will be seen that the reaction of the solutions between pH 4.0 and pH 5.0 was changed a good deal by the prolongation of the culture duration. As a result of this change of reaction, the absorption curve is somewhat different from Text-fig. 4. The curve of Text-fig 5 rose from pH 3.0 to the first maximum at pH 4.6–4.2, then it sank at pH 5.0–4.5 (plotted in the curve at pH 4.75). The difference from Text-fiig. 4 is that sinking at pH 5.0–4.5 in Text-fig. 5 is less significant when compared with that at pH 5.0 in the former. The cause of this difference is chiefly the change of reaction in the solution. The reaction of the solution having as its initial pH=5.0, shifted to pH 4.5. As will be seen from Experiment 21, the maximum absorption took place in the solution of pH-values between 4.5 and 4.4. Therefore one comes to a conclusion that the absorption in the solution of initial pH=5.0 was affected by the unfavourable reaction at first and the favourable at last, and this slight sinking in the absorption curve was the result of these two opposite influences.

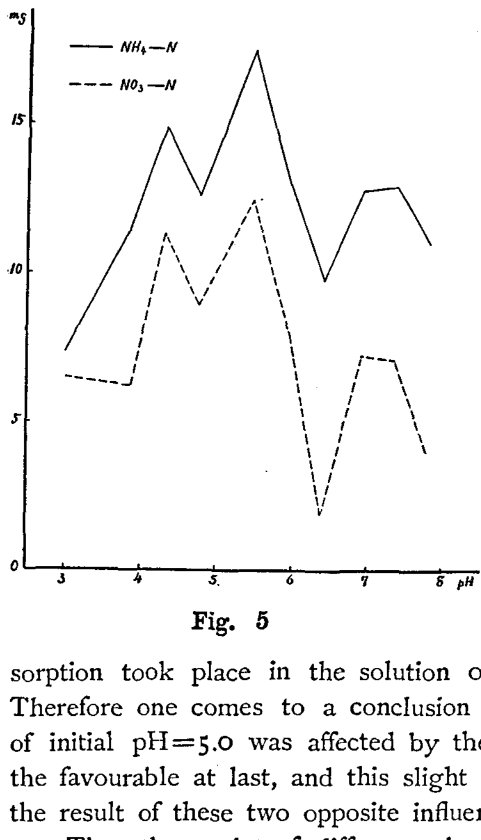


Fig. 5

The other point of difference in the form of the absorption curves between Text-fig. 4 and 5 is that in the former the second maximum occurred at pH 6.0, while in the latter at pH 5.5–5.4. The second maximum was unconstant due to unknown reasons. As will be seen later, sometimes equal absorption occurred at these pH-values, but usually the absorption in the solution of pH 6.0 was greater than in that of pH 5.5 or vice versa.

As in Experiment 21 the absorption of nitrate was better in the solutions having weak acid reaction and worse in the alkaline side. But no better absorption of ammonia in the alkaline solutions was to be recognized when compared with that in the acid solutions with pH-values 5.0–6.0. This might be the result of the effect of growth which became significant as the duration of the experiment was prolonged. It must be borne in mind that the pH-range 5–6 is very favourable for the growth of maize (WEIS, 1919 and LOO, 1927). When the duration of the experiment was short, the difference of growth between these solutions insignificant; but when it is long, the effect of growth of seedlings is not negligible, the better the growth, the greater the requirement of the nutrient salt.

In spite of these influences of change of reaction and plant growth, the form of absorption curves comparing these experiments was generally similar especially with respect to the minimum points. They were situated respectively in the vicinity of pH 5.6, 6.4 and 7.8. This result is worthy to note.

DECAPITATION

Experiment 23

The results of experiment 22 recorded above show clearly the effect of growth of the culture seedlings upon the absorption of ammonia and nitrate. In order to avoid this influence of growth, further experiments were carried out in which the decapitation of the culture seedlings was practiced. As soon as the seedlings were transferred from the preliminary culture to the culture solutions containing buffers, the upper part of the seedlings just below the cotyledon was cut away with scissors. The root system was held in the hole of the cover of the culture cylinder with cotton so that the cut end was always outside of the culture vessel. By thus treating, the development of bacteria on the cut end and the excretion of substances from the same place were completely obviated. The roots were left in the culture solutions for twenty four hours. After the experiment the external solution was analysed in order

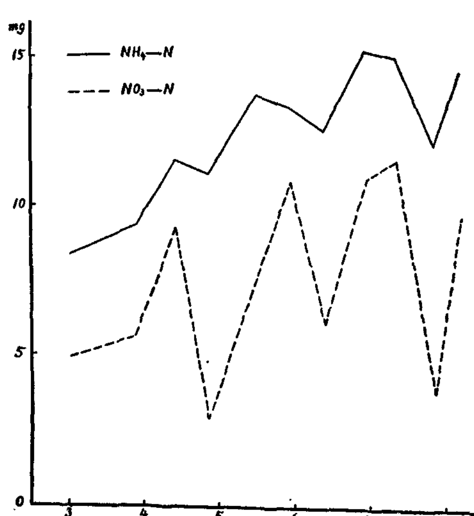


Fig. 6

to determine the absorption of nitrate and ammonia. Table 22 and Text-fig. 6 show the results of Experiment 23.

It will be seen from these data that the reaction of the culture solutions was changed a good deal in those having pH values 4.6–5.0 and 7.5–8.5. Though the duration of the experiment was just the same as in the foregoing experiment, the amount of salts absorbed was less in this case as the result of the decapitation. This is especially true in the solutions whose pH-values a favourable to the growth of maize, showing clearly the influence of growth of seedlings upon absorption. The form of the absorption curve is essentially the same as that of Experiment 21. The absorption of ammonia was the least in the solution of pH 3.0. As the acidity decreased the absorption increased gradually and reached the first maximum in the vicinity of pH 4.5. Then it sank slightly, but noticeably at pH 5.0–4.7. The second maximum was at pH 5.5 and a third at ca. pH 7.0 with two minima at pH 6.4 and ca. pH 7.9 respectively. Thus the maximum and minimum points were exactly coincident with those of the foregoing experiment. In general, the absorption of ammonia was better in the alkaline than in the acid side. The absorption of nitrate was parallel to that of ammonia; it rose with the maximum and sank with the minimum points of the absorption curve of ammonia. No greater absorption of nitrate in the acid than in the alkaline solution can be recorgnized. The amount of nitrate nitrogen absorbed in the solution of pH-value 5.5 was ca. 8 mg and of pH 6.0 11 mg, but on the alkaline side, almost the same or even a greater amount was taken up by the root system except in the solution of the initial pH 8.0. This phenomenon suggests the idea that the greater absorption of nitrate in the acid solution is not purely due to the influence of hydrogen ion concentration of the solution as usually claimed by many authors, but is at least partly due to the effect of the growth of the culture plants. In the present case, the effect of growth and assimilation was almost, if not completely, overcome by the decapitation, and consequently no more nitrate was absorbed in the acid than in the alkaline solution.

Experiment 24

Experiment 24 was carried out in the laboratory. It lasted only 6 hours. Five roots were cultured in the solution. The results of this experiment are recorded in Table 23 and illustrated in Text-fig. 7.

Table 23 shows that within this short experimental time almost no change of reaction took place in the culture solutions. On the other hand, the absorption of both ammonia and nitrate was also very insignificant; the greatest amount of ammonia absorbed was ca. 10 mg. of nitrogen in the solution of pH 8.5 and the least was only ca. 1.5 mg in the solution of pH 3.0. However, the influence of hydrogen ion concentration upon the absorption was evident. The absorption of ammonia became greater as the acidity of the solution decreased and the absorption of nitrate was always accompanied by that of ammonia. As in Experiment 23 no significant decrease of absorption of nitrate in the alkaline side could be discovered.

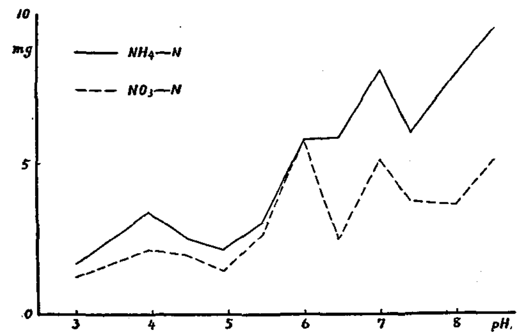


Fig. 7

The form of the absorption curve (Text-fig. 7) is somewhat different from that of the former experiments. First of all, the divergence between the absorption of ammonia and that of nitrate in the solutions more acid than pH 6.0 is very small. The absorption of ammonia and nitrate was almost equal in the solutions of pH 5.5 and pH 6.0. In the solutions less acid than pH 6.0, divergence of absorption became greater. Secondly there was no depression in the curve of absorption of ammonia at pH 6.4, the curve rose steadily from pH 5.0 and reached the seecond maximum at pH 6.0 as in the former experiments. But the absorption of ammonia at pH 6.4 was equally good as that at pH 6.0. Thirdly the curve of absorption of ammonia sank at ca. pH 7.5 instead of pH 7.8. Notwithstanding these variations the curve for absorption of ammonia agrees with that of the former experiments in the point that it is wave formed and has three maximum points at ca. pH 4.5, 6.0, 7.0 and three minimum points at ca. pH 5.0, 6.4 (?), and 7.4 respectively.

The curve of absorption of nitrate is more nearly coincident with that in the former experiment than the absorption curve of ammonia. The minimum points are obviously located at pH 5.0 and pH 6.4. The third minimum point occurred at pH 8.0, though this point of minimum is not quite evident, since the difference between the amount of nitrate nitrogen absorbed between solutions of pH 7.5 and pH 8.0 is within the range of the probable error.

Then the question arises as to what is the cause of the variation in the absorption curve of ammonia. The experimental condition of Experiment 24 was different from Experiment 23 in that the experimental time was shorter and the experiment was conducted in the laboratoly under room temperature. In order to ascertain the effect of the duration of the experiment upon the absorption of ammonia and nitrate by root system only, the following two experiments were carried out in the laboratory. Three roots were cultured in buffer solutions for 24 and 48 hours respectively. The results are shown in Tables 24 and 25, and the absorption curves in Text-figs. 8 and 9.

Experimeat 25

Let us consider first the results of Experiment 25 which was lasted 24 hours. Table 24 shows that the reaction of the solution changed a little in consequence of the prolongation of the experimental time. But the difference of absorption of amonia between the acid and alkaline solution became insignificant: the amount of ammonia absorbed was nearly 2×10^{-5} N in every solution except in the solutions of pH 5.0 and 5.5. The amount of ammoniacal nitrogen absorbed was from 10 to 13 mg. On the other hand, the difference of absorption of ammonia between solutions of pH 5.0 and its near regions became more significant, if compared with the result of the foregoing experiments. To speak in detail, in the case of shorter duration (6 hours) the difference of absorption of ammoniacal nitrogen between pH 5.0 and its near regions was only 1 mg, but it was more than 5 mg in the present case. The same relation holds good in the case of absorption in solutions of pH 6.4 and its near acidic regions. In the foregoing experiment, no depression in absorption of ammoniacal nitrogen was found at pH 6.4, but there was at least a slight indication of depression of absorption at pH 6.4 in this experiment, though the difference of absorption between pH 6.4 and its near regions was very insignificant. The third minimum point of absorption of ammonia occurred at pH 7.9–8.0 in this case.

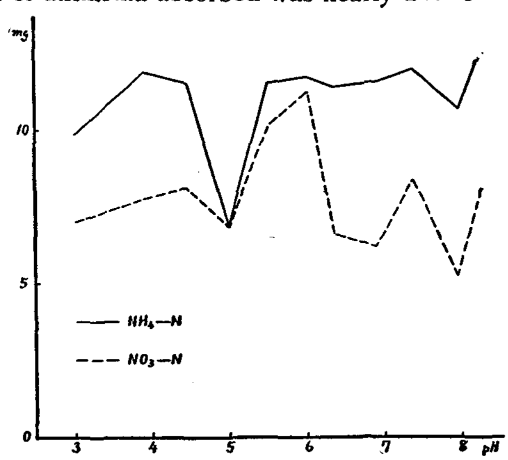


Fig. 8

The absorption of nitrate was essentially the same as in the foregoing experiment in every respect, showing that the absorption of nitrate was not affected by prolonging the duration of the experiment. It is worthy to note that within the pH-range 5.0 to 6.0, the divergence between the absorption of ammonia and nitrate was very insignificant. The ratio of absorption NH_3/NO_3 in this pH-ranges was practically eqnal to unity. This result agrees with the finding of Experiment 24. It is obvious, therefore, that the length of the duration of the experiment has no relation to this phenomenon.

Experiment 26

The data recorded in Table 25 shows the prolongation of the time duration of the experiment to 48 hours did not cause any increase of absorption of ammoniacal nitrogen. But on the contrary, a part of the ammoniacal nitrogen absorbed in the first 24 hours has been given up by the root system to the solution. A comparison of Table 25 with Table 24 makes clear the fact that with a few exceptions in the solutions of pH 6.0, and 80, in almost all solutions a part of the ammoniacal nitrogen absorbed was lost during the later 24 hours. This might be expected from the present experimental conditions. The ammoniacal nitrogen absorbed could not be transferred and assimilated in the plants by decapitation. Concequently the ammoniacal nitrogen absorbed was accumulated saturatedly in the root system within a short time, perhaps a little more than 24 hours in our case. After saturation, not only the absorption of ammonia was hindered, but also a part of it was given out by the root system, if left in the solution for a longer time. It is interesting to see, however, that the amount of nitrate nitrogen absorbed increased a little during the last 24 hours. This is apparently due to the slow penetration of nitrate into the root cells. The saturation of nitrate nitrogen in the root system will not be reached in 24 hours. Accordingly, it penetrated into the root continuously, though the absorption of ammoniacal nitrogen was hindered by saturation. Therefore, in the case of decapitation, the ratio of absorption NH_3/NO_3 would come nearer and nearer to unity as the duration of the experiment prolonged. Table 24 and 25 show that it is the case.

Text-fig. 9 shows that the curves of absorption of ammonia resembles those of Experiment 23. They resemble, too, those of the foregoing experiments, but differ from those in the respect that the minimum at pH 6.4 became more apparent. Thus, in this curve, there are three evident minimum points at pH 5.0, 6.4 and ca. pH 7.5 respectively, a result which is in coincidence with those of the former experiments.

The absorption curve of nitrate nitrogen is in fact the same as that of ammonia, though the third minimum point was at pH 7.9 instead of pH 7.4–7.2. Just as in the case of Experiments 24 and 25 the divergence of absorption between ammonia and nitrate in the solutions from pH 5.0 to 6.0 is very small; the ratio NH_3/NO_3 is equal to unity.

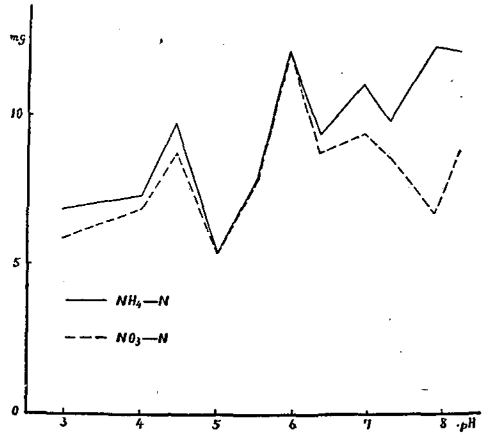


Fig. 9

A comparative examination of Text-figs. 7, 8 and 9 makes clear the fact that the variations in these curves of absorption of ammoniacal nitrogen were caused directly by the time factor and indirectly by the temperature factor of the experiment. The curve in Text-fig. 7 shows the result of absorptson in a short duration; the difference between maximum and minimum points was so insignificant that the minimum points were not remarkable. But when the experimental duration was prolonged to some extent, the absorption of ammoniacal nitrogen in the neighbourhood of minimum points increased and the minimum points became more and more evident. The form of the absorption curve in Experiment 26 is essentially the same as that in Experiment 23 which was conducted in the green house. No doubt, the relatively low temperature in the laboratory made it necessary for the root sistem to absorb the same amount of nitrogen in a relatively longer time. The form of absorption curve for nitrate nitrogen seems to have no great relation to the prolongation of the duration of the experiment. The amount of nitrogen absorbed increased gradually as the experiment went on, but the form of the absorption curves was the same, comparing those of the shortest and longest experimental duration. Another noteworthy point is the slight or no divergence between the absorption of ammonia and nitrate in the solution having pH-values from 5.0 to 6.0. The cause of this phenomenon is not clear, but it is possible that the effect of temperature has taken part in it. However, any definite conclusion requires further study.

The results of decapitation hitherto described may be summarized briefly as follows:

When the effect of the growth of seedlings was removed by decapitation, the change of reaction of the solutions was retarded to some extent. The absorption of ammoniacal nitrogen increased gradually as the acidity of the solution decreased. With the pH-range of 3.0–8.5, there were three points of depression at ca. pH 5.0, 6.4 and 7.9 (or 7.2), a result which was similar to that in the case of plant in the normal state. The form of absorption curve, however, was varied to some extent due to the effect of the duration of the experiments and low temperature. The absorption of nitrate nitrogen was in general like that of ammoniacal nitrogen, but the curve of absorption was affected by prolongation of the experimental time. Under the condition of room temperature, there was little or no divergence between the absorption of ammonia and nitrate in the experiments conducted in the green house.

The results of Experiments 21–26 agree with each other so far as the absorption curves of both ammoniacal and nitrate nitrogen are wave-shaped and as the points of depression are relatively fixed notwithstanding the variation of experimental conditions. As the pH-values of any of these solutions differ from their neighbours by the step of 0.5 of SÖRENSEN's unit, it is difficult to determine whether or not the depressions occur exactly at these points. In order to ascertain more precisely the situation of the points of depression, solutions prepared were ranged from pH 3.0 to pH 8.6 by the step of 0.2.(1) The method of perparation of the culture solutions was exactly the same as in the foregoing experiments. The composition of culture solutions containing buffer is shown below.

pH-value of solution	M/5 H_3PO_4 cc.	M/5 NaH_2PO_4 cc.	M/5 Na_2HPO_4 cc.	ca. 0.35 N NH_4NO_3 cc.	H_2O cc.	Sum cc.
3.0	10	90	0	10	890	1000
3.2	6	94	0	”	”	”
3.4	4	96	0	”	”	”
3.6	3	97	0	”	”	”
3.8	1.5	98.5	0	”	”	”
4.0	1	99	0	”	”	”
4.2	0.5	99.5	0	”	”	”
4.4	0.2	99.8	0	”	”	”
4.6	0	100	0	”	”	”
4.8	”	99.6	0.4	”	”	”
5.0	”	99.0	1.0	”	”	”

(1) With few exceptions in the range from pH 3.0 to 4.0 or from 8.0 to 8.6; these pH-values are thought to be unfit for the normal growth of most plants.

pH-value of solution	M/5 H_3PO_4 cc.	M/5 NaH_2PO_4 cc.	M/5 Na_2HPO_4 cc.	ca. 0.35 N NH_4NO_3 cc.	H_2O cc.	Sum cc.
5.2	0	98.5	1.5	10	890	1000
5.4	"	96.5	3.5	"	"	"
5.6	"	94.5	5.5	"	"	"
5.8	"	90.2	9.8	"	"	"
6.0	"	85	15	"	"	"
6.2	"	80	20	"	"	"
6.4	"	75	25	"	"	"
6.6	"	65	35	"	"	"
6.8	"	55	45	"	"	"
7.0	"	49.5	50.5	"	"	"
7.2	"	39.5	60.5	"	"	"
7.4	"	24	76	"	"	"
7.6	"	15	85	"	"	"
7.8	"	10	90	"	"	"
8.0	"	6.5	93.5	"	"	"
8.2	"	4	96	"	"	"
8.4	"	2	98	"	"	"
8.6	"	0	100	"	"	"

Experiment 27

Experiment 27 was also carried out in the laboratory. Three roots were grown in the culture solutions. As the temperature of the laboratory became quite high in June, the experiment lasted only 12 hours. Table 26 shows that the amount of nitrogen absorbed was almost equal to that in Experiment 26 which lasted 48 hours. Little or no change of reaction took place in these solutions. The absorption curve (Text-fig. 10) was practically the same as in Text-fig. 9; rising from pH 3.0 to the first maximum pH 4.0. Then it sank suddenly at pH 5.2-5.1. Because no experiment was

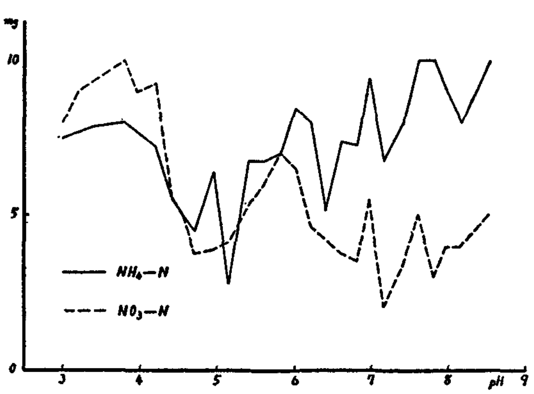


Fig 10

worked out with pH 5.2 and 5.4 in the foregoing cases, the first minimum has been always situated at pH 5.0–4.8, since the absorption at pH 5.5 was usually little greater than at pH 5.0. The second maximum was situated at pH 6.0 and the second minimum at pH 6.4, a result quite similar to that of the foregoing experiments. The curves rose again from pH 6.6 onward and reached a third maximum at pH 7.0. Then it depressed again at pH 7.2. The absorption curve of nitrate nitrogen sank at pH 7.8, but that of ammoniacal nitrogen did not. The latter sank at pH 8.1–8.2 instead of pH 7.8.

The absorption of ammonia and nitrate in the solution more acid than pH 6.0 was almost equal. This is in agreement with the finding of experiments which were conducted in the laboratory.

Experiment 28

Table 27 shows the results of Experiment 28 which was a repetition of the former. They agree in every respect with those of Experiment 27 and therefore need not to be described here. A glance at Text-fig. 11 will make clear the fact that the absorption curve is wave-shaped and possesses more than three minimum points at pH 5.2–5.1, 6.4, 7.2 and ca. 8.0 respectively.

Thus from the experiments using solutions having pH-range from 3.0 to 8.6 by a step of 0.2 instead of 0.5, curves of absorption with 4 minimum points were obtained. Among these, the first minimum at ca. pH 5.2 agrees with that at ca. pH 5.0 in the former experiments. The second minimum at pH 6.4 remained unchanged. Other minimum points at pH 7.2 and ca. pH 8.1, was recognized in the curves of the former cases. The minimum point at ca. pH 8.1, however, is but a variation of the point at pH 7.9. As to the minimum point at pH 7.2, this was not altogether unknown. When on occasions the reaction of solution having the initial pH 7.5 changed to pH 7.2, there would be a depression in the curve (for example, see Text-fig. 9). At any rate, the depressions

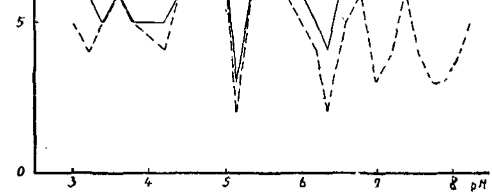


Fig. 11

of absorption curve in the solutions of alkaline reaction were relatively variable.

NORMAL PLANT

From the data of Experiments 21–28 it is clear that the fundamental results are essentially the same in both the normal and the decapitated plant, though a slight transformation of absorption curve has been recognized. As the amount of absorption in the case of decapitation was usually insignificant, it is advantageous to use a normal seedling as the indicator plant.

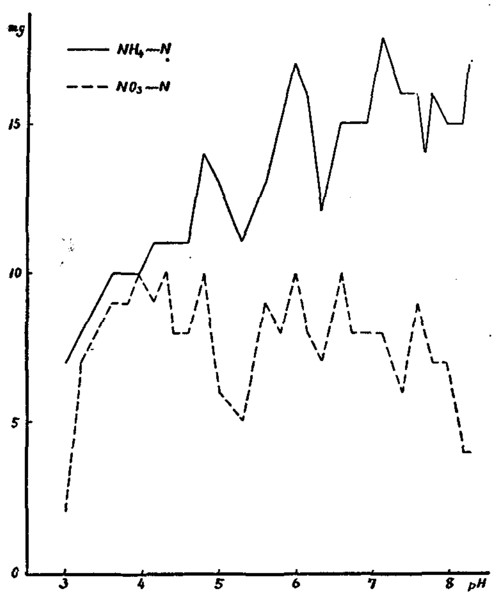


Fig. 12

Two experiments 29 and 30 with normal seedlings were carried out in the green house. The results of Experiment 29 are summarized in Table 28 and illustrated in Text-fiig. 12.

Experiment 29

The reaction of solutions ranged from pH 4.6 to 5.4 and that of solutions more alkaline than pH 8.0 changed a good deal during the course of the experiment. The effect of the change of reaction was apparent. The first minimum point appeared at the initial pH 5.4 instead of at the initial pH 5.2. Since the solution of pH 5.2 changed to pH 4.6 and that of pH 5.4 to pH 5.2, it is rather a matter of course to find the change of the point of depression from the initial pH 5.2 to the initial pH 5.4. Solution of pH 6.4 changed very slightly and this point of absorption curve was depressed as usual. On the alkaline side, the points of depression were not marked on account of the change of reaction. But when the absorption curve was plotted againts the average pH, a point of depression was obtained at pH 7.7 for ammoniacal nitrogen and another at pH 7.4 for nitrate. In short, the form of the absorption curves was similar to that of Text-fig. 5.

The absorption of ammoniacal nitrogen increased with the decrease of acidity of the solution and the reverse is true in the case of absorption of nitrate nitrogen, though in extremely acid solutions such as those of pH 3.0 and 3.2, the sbsorption of nitrate was very bad, perhaps due to the toxicity of high acidity. The absorption of ammoniacal and nitrate nitrogen in the solutions ranging from pH 3.2 to pH 4.4 was almost equal; the ratio of absorption NH_3/NO_3 was always near to unity. But from pH 3.6 on, the divergence between the absorption of ammonia and nitrate became greater and greater as the reaction of the solution became less and less acid. No contact of curves of absorption of ammonia and nitrate occurred in the pH-range of 5.0–6.0 which was always the case in the experiments conducted in the laboratory.

Experiment 30

Experiment 30 was performed as a repetition of Exp. 29, 4 seedlings were grown in solutions for 24 hours. The results are shown in Table 29 and Text-fig. 13.

Though the form of absorption curves is slightly different from Text-fig. 12, the essential points are in coincidence with each other. The absorption curve was depressed at pH 5.1. This point was different from that of Text-fig. 12 where the first minimum was situated at pH 5.4–5.2. Perhaps the real point of the depression was pH 5.2. In Experiment 29 the reaction of the solution having the initial pH 5.2 changed to pH 4.8; the later pH-value is a point too distant from the former which is unfavourable for absorption, and therefore pH 5.4–5.2 became the minimum. But in the present case, the reaction of solution of pH 5.2 changed only slightly, consequently the absorption at this point remained as minimum. The reaction of the solution with the initial pH 5.4

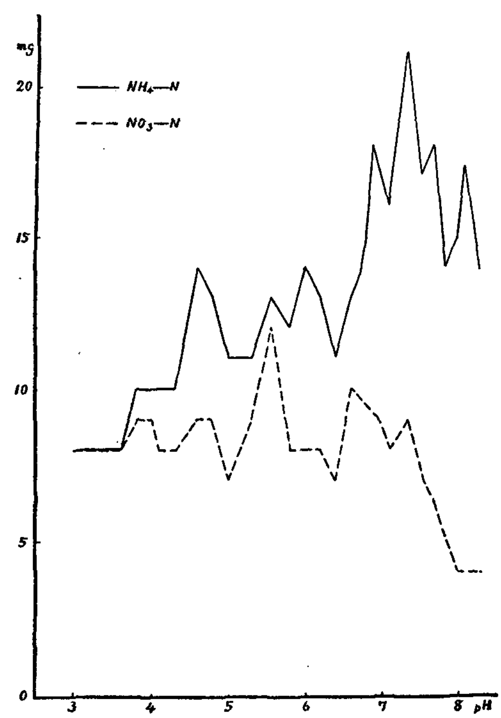


Fig. 13

changed to 5.2 as in the foregoing experiment and the absorption at that point was almost equally poor as that in the solution of pH 5.2–5.0. Other points of depression were situated at pH 6.4, 7.1 (for absorption of nitrate) and 7.9 (for absorption of ammonia). These were almost coincident with those of Experiment 29. Moreover, the contact of curves of absorption for ammonia and for nitrate coincide in the pH-range 3.0–4.4; the superior absorption of ammonia and the inferior absorption of nitrate in alkaline solutions were also found in the results of this experiment.

The above results show clearly that the difference between normal and decapitated plant in relation to the influence of hydrogen ion concentration upon the absorption has to do with the effect of growth. In the case of normal plant, the amount of nitrate absorbed of usually greater in the weakly acid side than in the alkaline side. The divergence of absorption between ammonia and nitrate is more significant in the case of normal plant than in the case of the decapitated. But as to the wave form of absorption curve and the situation of points of depression in the curve, they were fairly coincident in both cases.

2. *Experiments with uniform amount of cations in buffer*

In Experiments 21–30, sodium phosphate mixture was employed as buffer in which the amount of anions was equal in every solution. The amount of sodium ions, however, in the solutions of most alkaline reaction was almost two times that in the extremely acid solutions. That the different amount of sodium ion in different solutions may exert varying influence upon the absorption of ammonia and nitrate is a thing which might be expected from the important action of cations upon absorption. Therefore it could not be free from objection to ascribe the wave-fromed curve of absorption alone to the influence of hydrogen ion concentration of the solution with such buffer. Accordingly, sodium phosphate mixture with uniform amount of cations was prepared in the following way. A definite amount of distilled water was added to the stock solution of sodium phosphate mixture so that the amount of sodium ions was equal in every mixture. 100 cc. of the buffer mixture thus prepared and 10 cc. of ca. 0.035 N solution of ammonium nitrate were taken in a measuring flask and diluted to 1000 cc. In this case, the pH-values of the buffer mixture were almost unchanged by dilution.

Experiment 31

Experiment 31 was performed in the laboratory, and the experimental duration was 22 hours. Four seedlings were cultured in the solutions. Tabel 30 shows the results of this experiment.

From the data recorded in Table 30, it will be seen that the essential results were in agreement with those of experiments with uniform amount of phosphates; that is to say the absorption of ammonia decreased with the increase of acidity, while that of nitrate showed the reverse relation. The absorption curve was wave-shaped having more than three points of depression (Text-fig. 14). Of course, the minute datails of the results may have more or less variation as might be expected from the different ionic conditions in the buffer solution. For example, the second point of depression was located at pH 6.2 instead of 6.4 and the depression in the alkaline side were not clear. The latter fact has been observed in the experiments with normal plants, but in the present case it was more obvious. The divergence of absorption between ammonia and nitrate was very great in the alkaline solution, showing that the absorption of ammonia in the alkaline solutions had been hindered to some extent by the excess of sodium ions in the former cases.

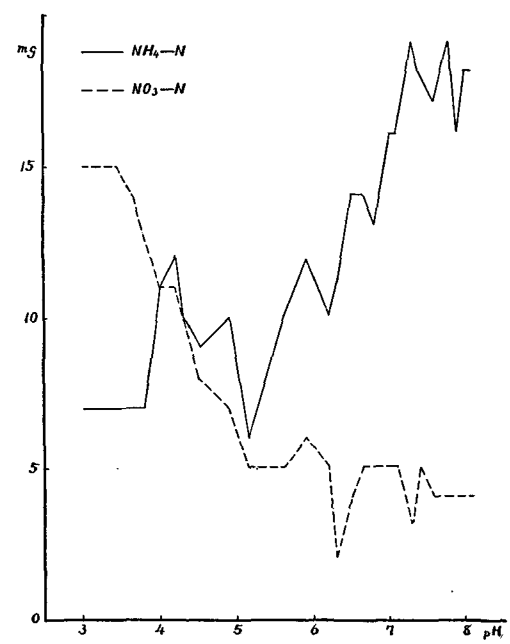


Fig. 14

The absorption of nitrate in the pH-range 3.2–3.8 was slightly greater than the absorption of ammonia. As a result of this difference of absorption, the reaction of these solutions became a little less acid. In these pH-ranges one usually found almost equal absorption of ammonia and nitrate, though occasionally more nitrate was absorbed.

Experiment 32

Experiment 32 was a repetition of Experiment 31. It was performed in the green house. Six seedlings were cultured in the buffer solutions for 24 hours. Table 31 and Text-fig. 15 show the results of this experiment.

During this experiment, the temperature was comparatively low in the green house, being 15°–20° C. Consequently the absorption was less than that in Experiment 31, in spite of the longer experimental duration. From Tble 31, it will be clear that the results of this experiment were chiefly coincident with the former one; the only difference is that the fluctuation of the curves (Text-fig. 15) is relatively small as a result of the inferior absorption due to low temperature. As to the points of depression, they were situated in almost the same positions as in the foregoing experiments.

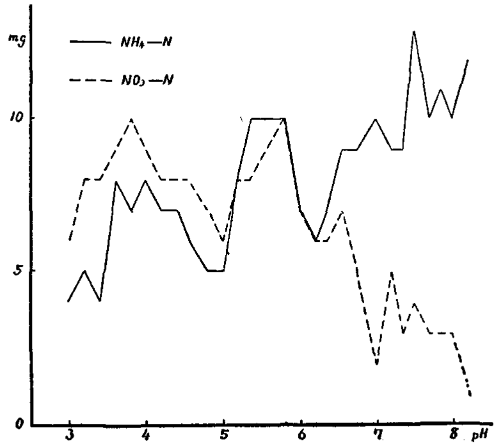


Fig. 15

Thus from the results of the *foregoing two experiments, it is* obvious that the absorption of ammonia and nitrate in the sodium phosphate mixture having uniform amount of cations, was in general similar to that having uniform amount of anions. The divergence of absorption between ammonia and nitrate on the alkaline side may be exaggerated to some extent by removing the comparative excess of sodium ions, and the depression on the alkaline side may become less evident. But that there were more than three points of depression in the absorption curves in the pH-range from 3.0 to 8.6 was common in both cases. These points were situated in the vicinity of pH 5.2, 6.4, 7.2 and 7.9. They occupied relatively definite positions in the curve under varied experimental conditions. But before we go further into the significance of this phenomenon, let us see whether similar results can be obtained with other kinds of buffer solutions.

B. Oxalate mixtures

Two experiments with sodium oxalate mixture were carried out, one having a uniform amount of cations and the other of anions. As externally acting oxalate ion is toxic to plant cells and the existence of organic matter in the solution is liable to contamination by microorganisms, care has been taken in choosing the concentration of the buffer solution and suitable length of experimental duration. Both experiments lasted 24 hours.

1. *Experiment with uniform amount of cations in buffer*

Experiment 33

The buffer solutions were prepared by titrating a mixture of sodium hydroxide and sodium oxalate with N/10 oxalic acid. Their composition, in detail was as follows:

pH of solution	N/10 Sodium oxalate cc.	N/10 NaOH cc.	ca. 0.035 N NH_4NO_3 cc.	N/10 Oxalic acid cc.	H_2O cc.	Sum cc.
3.5	10	20	10	20	940	1000
4.0	"	"	"	15	945	"
4.2	"	"	"	13	947	"
4.5	"	"	"	12	948	"
4.8	"	"	"	11	949	"
5.0	"	"	"	10.7	949.3	"
5.2	"	"	"	10.4	649.6	"
5.4	"	"	"	10.2	949.8	"
5.6	"	"	"	10.1	949.9	"
5.8	"	"	"	10	950	"
6.0	"	"	"	9.94	950.06	"
6.2	"	"	"	9.8	950.2	"
6.4	"	"	"	9.76	950 24	"
6.6	"	"	"	9.74	950.26	"
6.8	"	"	"	9.7	950.3	"
7.0	"	"	"	9.6	950.4	"
7.8	"	"	"	9	951	"
8.4	"	"	"	8.5	951.5	"
9.0	"	"	"	6	954	"

It is clear from the above Table that the buffer power of this mixture was very weak, especially in the pH-range from 5.6 to 7.2. In fact, the reaction of these solutions changed considerably during the experimental duration as will be seen from the results of Experiment 33 which are shown in Table 32.

Six seedlings were cultured in the buffer solutions. During this experimental duration, the temperature in the green house was very low.

Owing to this low temperature, no microorganisms were developed in the solutions. The culture solutions remained clear throughout the whole experiment. No unsound symptom which might be ascribed to the toxicity of oxalate appeared in the culture plants. But in general, the absorption was poor. The absorption curves of ammonia and nitrate came to contact with each other in the pH-range 4.5–5.5, a phenomenon characteristic to the culture at low temperature.

From pH 3.5 to pH 5.4, the reaction of the solution remained almost constant. It changed a great deal in the solutions less acid than pH 5.4; for example, the initial pH 6.4 of a solutions changed to 5.8, pH 7.2 to 5.8, and pH 8.4 to 6.4 etc. In these solutions, the absorption was affected by both favourbale and unfavourable reaction of the solution and it is very difficult to judge which pH-value was responsible for the results.

For the sake of convenience, the absorption curves were plotted against average pH-values. Text-fig. 16 shows that absorption of ammonia was inversely proportional to the acidity of the solution, the greater the acidity the less the amount of ammonia absorbed. The absorption of nitrate, however, was greater in the weakly acid side than in the alkaline side, thus causing a great divergence between the absorption of ammonia and that of nitrate. This is a result which is in common with sodium phosphate mixture.

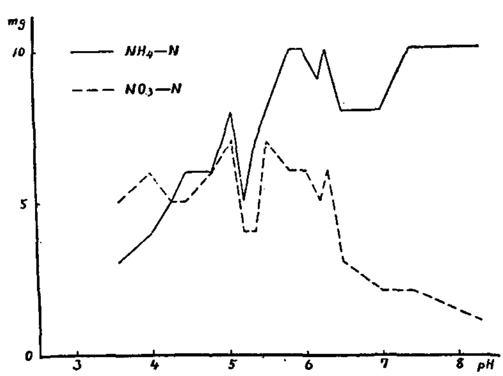


Fig. 16

From Text-fig. 16 it will be seen that a clear depression took place at pH 5.2. The curve rose from pH 5.4 to a maximum point at pH 6.0. Then it depressed again in the vicinity of pH 6.5. The absorption curve of ammonia rose again in the vicinity of pH 7.5, but that of nitrate depressed for good and all. Thus we have one depression at pH 5.2 and at least an indication of another at ca. 6.5. The other points of depression which have been found in the alkaline side were not found in this case, apparently due to the rapid change of reaction.

2. *Experiment with uniform amount of anions in buffer*

Experiment 34

The buffer solutions were prepared by adding a definite amount of N/10 sodium hydroxide to the mixture of oxalic acid and sodium oxalate till the required pH-value was obtained. In order to avoid the great change of reaction in the solution, the concentration of oxalic acid and sodium oxalate was increased almost twice as great as in the foregoing case. The following Table shows the composition of the buffer solutions.

pH	N/10 Sodium oxalate cc.	N/10 Oxalic acid cc.	ca. 0.035 N NH_4NO_3 cc.	N/10 NaOH cc.	H_2O cc.	Sum cc.
4.2	20	20	10	30	920	1000
4.4	"	"	"	33	917	"
4.6	"	"	"	35	915	"
4.8	"	"	"	36	914	"
5.0	"	"	"	37.1	912.9	"
5.2	"	"	"	38.5	911.5	"
5.4	"	"	"	39.4	910.6	"
5.6	"	"	"	39.6	910.4	"
6.0	"	"	"	40	910	"
6.4	"	"	"	40.2	909.8	"
6.6	"	"	"	40.5	909.5	"
6.8	"	"	"	40.6	909.4	"
7.0	"	"	"	41	909	"
7.3	"	"	"	41.2	908.8	"
7.6	"	"	"	41.8	908.2	"
8.0	"	"	"	42	908	"
8.5	"	"	"	45	905	"

Four seedlings were grown in each culture solution. The results of Experiment 34 are shown in Table 33 and Text-fig. 17.

The reaction of the solutions between pH 5.4 and 6.0 became less acid during the experiment. Since there was no greater absorption of nitrate than ammonia and no development of bacteria in the solution, perhaps the penetration of oxalate ions was the cause of this decrease of acidity. The reaction of the solutions less acid than pH 6.4, however, became more acid as usual. But the change of reaction in the alkaline solutions was not so remarkable as in the foregoing experiment, with the exception of three most alkaline solutions, the difference between initial and the final pH-values being not more than 0.3.

Text-fiig. 17 shows the absorption curves of this experiment. From pH 4.2 to pH 6.0, they resembled those in Text-fig. 16 in every respect. The first depression was also situated at pH 5.2. The curves rose from pH 5.2, reached a maximum at ca. pH. 6.1, and then suddenly sank at pH 6.4. Two other depressions in the curve of absorption of ammonia were situated respectively at pH 7.2 and pH 7.8. The sbsorption curve for nitrate, however, sank forever from the neutral point.

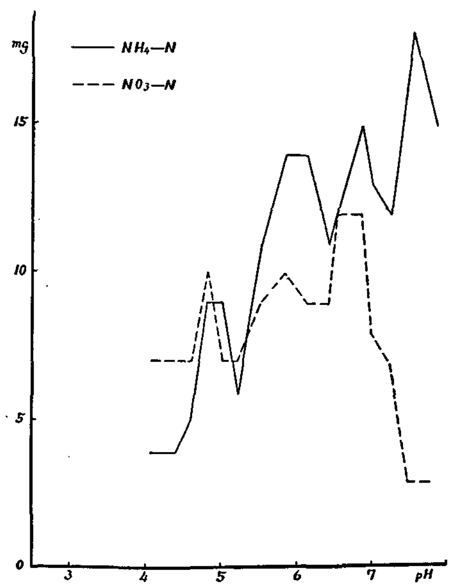


Fig. 17

The form of the absorption curves in Txet-fig. 17 differed from that in Text-fig. 16 in that there were 4 points of depression in the former, but only two in the latter. Whether or not the depression of curve in alkaline side is due to the excess of sodium ions is a matter of doubt. The writer is of the opinion that the disappearance of points of depression in the alkaline side of the absorption curves in Text-fig. 16 was caused by the remarkable change of reaction in these solution rather than by the removing of the excess of sodium ions. The results of Experiments 31 and 32 with equal amount of cations in sodium phosphate mixtures furnish proof of the truth of this statement.

Therefore, it may be concluded that the results of experiments with oxalate mixture are essentially the same as those with phosphate mixture. In both experiments with buffer solutions having uniform amount of cations and the same amount of anions, there were evidently two points of depression in the absorption curves situated respectively in the vicinity of pH 5.2 and pH. 6.4. The other points of depression were found at ca. pH 7.2 and ca. pH 7.8 in the case of uniform amount of anions, but not found in the case of uniform amount of cations owing to the remarkable change of reaction in the alkaline solutions. In other words, the results are in general agreement with those in the experiments with phosphate mixtures.

C. Complete solutions plus sodium phosphate mixtures

The results of experiments described thus far have all dealt with incomplete solutions. The absorption of nutrients by plants in such solutions may differ from that in the complete solutions. It is necessary, therefore, to study the influence of hydrogen ion concentration upon the absorption in a complete solution where all salts necessary for the development of plants are present in the balanced condition. For this purpose, a series of complete solutions containing sodium phosphate mixture with uniform amount of anions was prepared and two experiments were performed with these solutons for a period of 48 and 72 hours respectively. Six seedlings were grown in each solution. The composition of the solutions is shown in the following Table:

pH	Stock solution (cc.)		M/5 H_3PO_4	M/5 NaH_2PO_4	M/5 NaH_2PO_4	H_2O	Sum
	A	B (NH_4NO_3)	cc.	cc.	cc.	cc.	cc.
3.0	25	25	10	90	0	850	100
3.4	,,	,,	4	96	0	,,	,,
3.8	,,	,,	2 5	98.5	0	,,	,,
4.3	,,	,,	0	100	0	,,	,,
4.8	,,	,,	,,	99.2	0.8	,,	,,
5.0	,,	,,	,,	98	2	,,	,,
5.2	,,	,,	,,	97	3	,,	,,
5.4	,,	,,	,,	96	4	,,	,,
5.6	,,	,,	,,	93	7	,,	,,
5.8	,,	,,	,,	90	10	,,	,,
6.0	,,	,,	,,	85	15	,,	,,
6.2	,,	,,	,,	80	20	,,	,,
6.4	,,	,,	,,	70	30	,,	,,
6.6	,,	,,	,,	60	40	,,	,,
6.8	,,	,,	,,	50	50	,,	,,
7.0	,,	,,	,,	40	60	,,	,,
7.2	,,	,,	,,	20	80	,,	,,
7.5	,,	,,	,,	10	90	,,	,,
7.8	,,	,,	,,	0	100	,,	,,

Iron was used in the form of $FeCl_3$, 2 drops of its 2 % solution being added to each litre of the culture solution.

Experiment 35

Table 34 shows the results of Experiment 35. It is obvious that the absorption of ammonia and nitrate in complete solutions was more favourable than that in the incomplete solutions. The general tendency of absorption, however, is the same between solutions complete and incomplete. That is, the absorption of ammonia was greater in the alkaline than in the acid side, while that of nitrate was better in the weakly acid solutions.

The absorption curves are illustrated in Text-fig. 18. The amount of ammonia absorbed increased with the decrease of acidity and reached a maximum at ca. pH 4.5. Then the curve sank gradually to the first minimum at pH 5.2–5.4. The amount of ammonia absorbed in the solution of the initial pH 5.2 was less than in its neighbours. This was apparently the result of small surface of the root, since the dry weight of the root in the solution was also less than that its neighbours. By dividing the amount of ammoniacal nitrogen absorbed by the dry weight, we found that it was greater than that in the solution of the initial pH 5.4. Accordingly the amount of ammoniacal nitrogen absorbed in the solution of the initial pH 5.4 is the minimum. Since the reaction of this solution changed to pH 5.2, the first minimum in this curve was not very different from that in the former experiments. It is interesting to see that the amount of the ammoniacal nitrogen absorbed in the solution having the initial pH 5.6 and final pH 5.4 was almost as poor as that in the solution of pH 5.4–5.2.

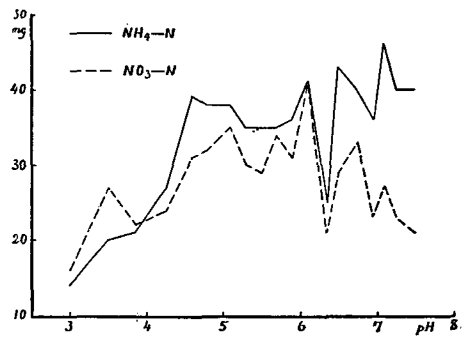


Fig. 18

The absorption curve ascended again at pH 6.2–6.1, but suddenly dropped at pH 6.4–6.3, which is a point of depression usually found in the foregoing experiments. This point of depression is the most obvious in this curve. Other such points were situated at pH 7.0. Because no experiment was made with solutions more alkaline than pH 7.8 which change to pH 7.1 during the experiment, another point of depression in the vicinity of 8.0 could not be ascertained.

The absorption curve for nitrate was in general parallel to that of ammonia, the only difference was that the first depression in the curve of absorption of nitrate was situated at pH 5.6–5.4 instead of at pH 5.4–5.2, as in the case of ammonia.

Experiment 36

Experiment 36 lasted 72 hours instead of 48. Its results are summarized in Table 35 and illustrated in Text-fig. 19.

It is clear from Text-fig. 19 that the curves of absorption differ from those in the foregoing experiment in many points. Firstly the first maximum point shifted from ca. pH 4.5 to ca. pH 4.0 (Text-fiig. 19) and the first minimum from pH 5.4 to pH 5.2. Secondly the second point of depression was situated at pH 6.1 instead of pH 6.3 and the degree of depression became less significant. Thirdly and lastly the amount of absorption became less in the zone of pH 4.8–5.4 (initial values) and greater in the zone more acid than pH 4.8 and less acid than pH 5.4, when compared with the amount absorbed during the first 48 hours. What is the cause of these differences?

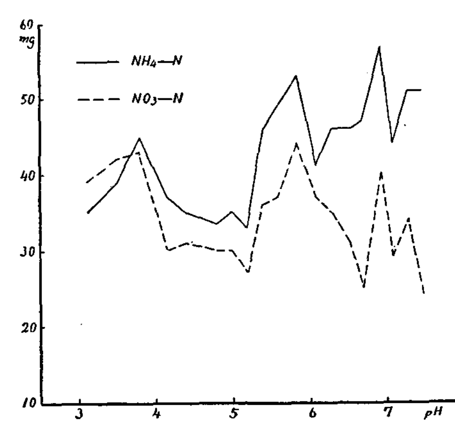


Fig. 19

Besides the change of reaction in the solutions, the influence of growth of the seedlings should be taken into consideration. A comparison of Table 34 with Table 35 will make clear the fact that the amount of ammonia and nitrate absorbed during 72 hours was in fact greater than that during 48 hours. But that the degree of increase of absorption was greater in the zone more acid than pH 4.8 and less in the other zone is perhaps due to the saturation of ammonia and nitrate in the plant body during the first 48 hours. The growth of seedlings in the later 24 hours of Experiment 36 was, on the contrary, especially good in the zone of pH 4.8–5.4, while no remarkable growth took place in solutions less acid than pH 5.4 and practically no growth in the solutions more acid than pH 4.8. This caused the shifting of the first maximum from pH 4.8 to pH 4.0 and the decrease of the amount of nitrogen absorbed in the zone between the initial pH 4.8 and 5.4.

From Text-figs. 18 and 19, it will be seen that absorption was vigorous at the pH-range from 4.5 to 5.0 in the first 48 hours, but in the following 24 hours this zone of vigorous absorption shifted to a zone of pH-values from pH 5.5 to pH 7.0. The replacement of the first and second point of depression seemed to have close relation to this shifting of favourable absorption.

In spite of these variations the results of these two experiments were fairly coincident in the point that the absorption of ammonia increased when the acidity of the solutions decreased and vice versa in the case of absorption of nitrate, and that the absorption curves possess 3 points of depression within the pH-range of 3.0 to 7.8 situated respectively at the vicinity of pH 5.2, 6.4 add 7.0. These are the same results obtained in the experiments with both sodium phosphate and sodium oxalate mixtures.

D. Experiments of longer experimental duration

Experiments with sodium phosphate, sodium oxalate, and complete solution + sodium phosphate mixtures resulted in the general resemblance, that in both cases of uniform amount of cations and anions in the buffer solutions, the absorption of ammonia was greater in the more alkaline than in the acid side and that the absorption curves possessed four points of depression situated in the vicinity of pH 5.2, 6.4, 7.0 and 7.8. In these experiments the duration was varied from 6 to 72 hours. In the case of longer duration, it was found that some variations took place in the form of the absorption curves. The cause of this variation has been ascribed to the influence of growth of seedlings. Besides the influence of growth, the change of colloidal properties of the protoplasm caused by the action of ammonia and nitrate should be taken into consideration. For the colloidal properties of the protoplasm of root cell may be changed not only by the hydrogen ion concentration of the external solution but also by the amount of ammonia and nitrate absorbed. The more they were absorbed in excess, the more serious may be this change.

Another point which requires further research is the relation between absorption and growth of the seedlings. It is a matter of question whether good growth is always accompanied by greater absorption or vice versa. According to TRUE and BARTLETT (1916), the greatest absorption of salts occurs in the most favorable solutions. On the other hand, ARRHENIUS (1922) found that in his experiments on the absorption of salts by wheat and radish the maxima of growth are correlated with minima of absorption curves. In explaining the cause of this phenomenon, ARRHENIUS stated: "Das Minimum im Wachstum auf Schäden in der Wurzel hindeutete, was seinerseites eine abnorme Salzaufnahme verursachen sollte." (ARRHENIUS, 1926; S. 59). It is really difficult to understand how the loss of normal permeability in the root cell increased the absorption instead of exosmosis of salts. In the first part of this work, we have seen that the injury of high acidity always causes the retardation of absorption. And indeed in some cases, the diffusing out of ammonia which has been absorbed by the roots took place when the root tissue began to decay.

But the duration of the experiments recorded above was not long enough and the seedlings which have been brought to a certain degree of deplopment were to insensible for any difinite conclution to be drawn from them. Accordingly experimets were performed with seedlings having shoots ouly 2 or 3 cm. long and without any trace of lateral root. Care has been taken in selecting seedlings of uniform development. They were washed clean with tap and distilled water and grown directly in the culture solutions without preliminary culture. The experiments lasted two weeks in the one and eleven days in the other. In order to prevent any change of reaction in the solutions during the course of experiment and to see the variation of absorption in a given interval of experiment, the culture solutions were renewed at definite intervals and the external solutions were analysed for ammonia only at the end of every renewal. The change of pH-value in all solutions was determinated at each renewal and the growth feature, especially that of the roots, was also observed carefully every day.

1. *Unbalanced solutions*

Experiment 37

Experiment 37 was performed with young seedlings of *Zea Mays*, 6 plants being grown in each culture containing 350 cc. of unbalanced solutions. The solutions used in this experiment were a mixture of sodium phosphate (with uniform amount of anions) and ammonium nitrate, the composition of which were exacty the same as those used in Experiment 27.

The growth of seedlings in the unbalanced solutions was exceedingly poor. In tne first week, no growth of lateral root was recognized in the solutions more acid than pH 5.0 and less acid than pH 6.0. As the experiment went on, a trace of lateral root could be found in the solution of these pH-ranges, but no growth whatever had taken place in the root of seedlings grown in the solutions of pH 3.0, 8.0 and 8.6 even at the end of the experiment. In general, the growth of lateral root in the solutions of pH-values from 4.0 to 6.6 was comparatively good, though the greatest length of the rateral root did not exceed 5 cm. The lateral root in the solutions ranged from pH 4.6 to 5.6 was long and fine, while that in solutions having pH-valuse 5.8–6.6 was short and blunt.

Three days after the beginning of the experiment, the colour of the root began to change. At first, a yellowish gray colour appeared on the surface of roots grown in the extremely alkaline solutions. This colour deepened with the progress of the experiment. It appeared on the roots in the solutions less alkaline at the end of the experiment. The roots in the solutions as acid as pH 5.8 became a little yellow and those in the extremely alkaline solutions became grayish brown. The roots in the solutions more acid than pH 5.0 remained white, though those in the most acid solutions became slightly pink.

The roots in both extremely acid and extremely alkaline solutions began ta decay as the experiment drew near the end.

The growth of the shoot was better in the acid than in the alkaline side. The shoots in the solutions more alkaline than pH 7.0 practically did not grow; they wilted one after another in the order directly proportional to the alkalinity. On the acid side, however, the shoot grew more or less favourably throughout the experiment with only one exception in the solution of pH 3.0 where the shoot wilted at the end of the experiment. The most favourable growth of shoot took place in the pH-range 4.8–6.6. In this pH-range, the growth of seedlings, including both shoot and root, in the solution of initial pH 5.4, 6.2 and 6.4 was apparently inferior to that of their neighbours. The dry weight of shoot and root of the seedlings recorded in Table 36a shows this fact clearly. Text-fig. 20 is the growth curve based on the results of Table 36a. When the dry weight of shoot or root was plotted

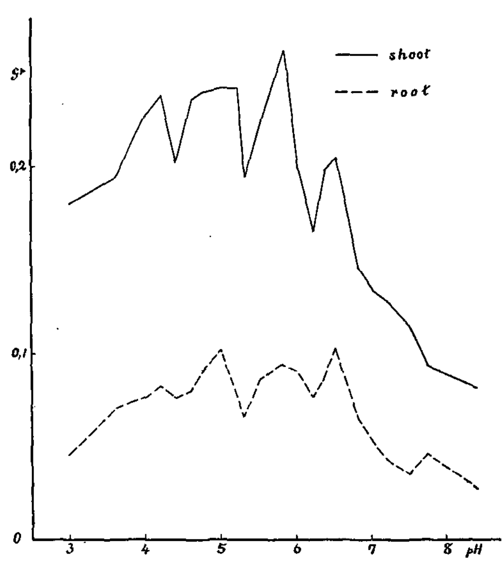


Fig. 20

against pH, curves having two minima situated at ca. pH 5.3 and ca. pH 6.3 were obtained.

Table 36a shows the change of reaction and the growth of seedlings in the solutions. From this Table, it will be seen that with few exceptions the reaction of the solutions was kept fairly constant by renewal. Great change of reaction took place in only two solutions having initial pH 5.0 and 5.2. The reaction of the initial pH 5.4 changed to pH 5.2 in the first 10 days, but remained unchanged during the later part of the experiment.

The amount of ammonia absorbed is shown in Table 36b. It is clear that the absorption varied at every renewal of cultute solutions. At the end of the first three days, the absorption of ammonia was greater on the weakly acid and alkaline side than in the solutions more acid than pH 5.0. This superior absorption in the weakly acid and alkaline solutions could not be found in the following three days. That is, from the third to sixth day, the amount of absorption in the solutions more acid than pH 3.0 increased to a certain degree, but that in solutions less acid than pH 6.0 decreased. And the amount of decrease is proportional to the alkalinity of the solutions, the greater the alkalinity in the solution, the greater the decrease of absorption. The absorption was hindered also from the 10th day on in the extremely acid solution and was wholly retarded at the end of the experiment in the solutions more acid than pH 4.6. In general, the absorption power of the seedlings in all solutions was more or less hindered by unfavourable growth as the experiment drew near the end, and in the last two days, there was almost no absorption in every solution.

When the amount of ammonia absorbed in the first three days was plotted against pH, a curve of three minimum points was obtained (Text-fig. 21). The points of depression were situated at pH 5.4–5.2, pH 6.4 and pH 7.0–7.2 respectively. Since the seedlings employed in the experiment were very uniform in development and no growth took place within the first 3 days, it may be assumed that the surface area of the root in different solutions was nearly equal to each other. Consequently the form

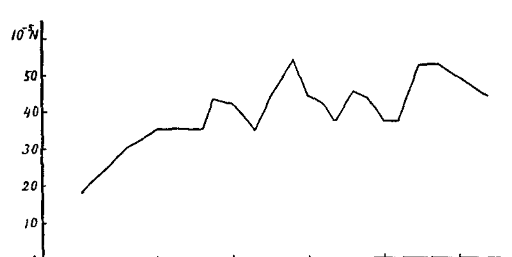
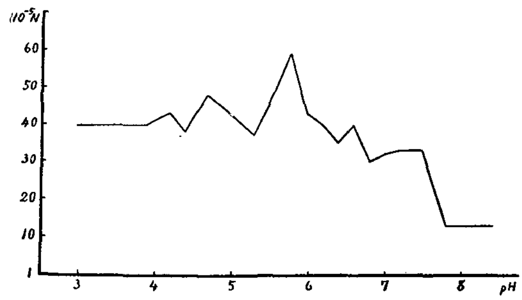


Fig. 21

of the absorption curve must be the same whether the amount of ammonia absorbed was divided by the dry weight of root or not. But as the experiment went on, a difference appeared in the growth of seedlings and the form of the absorption curve changed gradually. Text-fig. 22 shows the amount of ammonia absorbed in each cultures during the second three days. In this curve, the point of depression at pH 7.0–7.2 disappeared on account of the unfavourable growth in the alkaline solutions. Thus the manner of absorption changed with progress of the experiment till the last few days, the absorption was far from normal. Of course no point of depression could be found at pH 5.4–5.2, pH 6.4 and pH 7.0–7.2. But when the total amount of ammonia absorbed was divided by the dry weight of the root system, three evident depressions of absorption occurred respectively at pH 5.2, 6.4 and 8.0–7.8, (Text-fig. 23) a result which is in entire harmony with the findings of the foregoing experiments. It is also interesting to notice from Text-fiig. 23 that though the growth in the alkaline solutions was very bad, the amount of ammonia absorbed per one gm. of dry weight of the root was overwhelmingly greater than that in the acid side, showing that the absorption of ammonia on alkaline solutions was always better than in the acid ones.



Fgi. 22

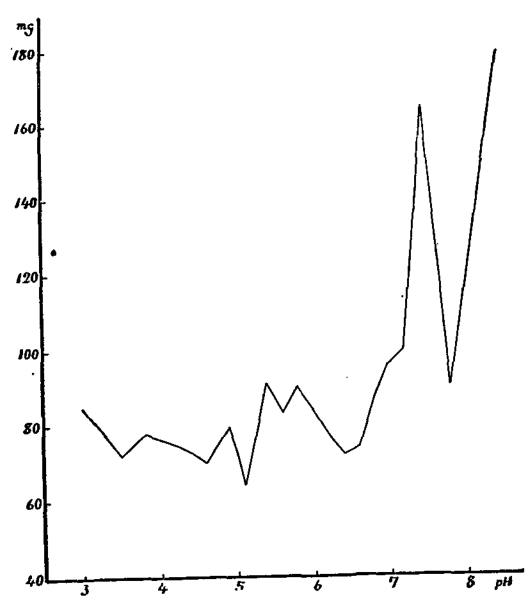


Fig. 23

2. *Balanced solutions*

Experiment 38

The results of the foregoing experiment shows that growth in an unbalanced solution is not normal. It is not sufficient to see the mutual relation of absorption and growth of plants from such results. Accordingly an experiment with complete solutions buffered by sodium phosphate mixture was performed. The composition of the culture solution was the same as that used in Experiment 35, except that the concentration of sodium phosphate in this case was just twice as that in the previous one. Six young seedlings of uniform development were grown in culture solution which were renewed every day. The experiment lasted 11 days.

The growth feature in this experiment was quite different from that of Experiment 37. There was no visible development of lateral roots in any solution at the end of the first day, but from the second day on, there were distinct differences in growth of both shoot and root amongst the seedlings in the solutions. Geneally speaking, the growth was better on the weakly acid and weakly alkaline side, the growth in the solutions ranging from pH 5.0 to pH 6.8 being the most favourable. Among these, the growth of lateral root in the solutions of pH 5.2 and pH 6.4 was obviously inferior that in their neighbouring solutions. But this phenomenon did not last long. A few days later, almost no difference could be observed except in the solution of pH 6.0 where the growth was remarkably inferior. As in the foregoing experiment, the growth feature of lateral roots between alkaline and acid solutions was different, the former was short and energetic, while the latter was long and fine. The difference in colour of lateral roots grown in acid or alkaline solution was also recognizable: those in the solutions more acid than pH 5.6 were white at first and became a little pink at last, but those in the solutions less acid than pH 5.6 were yellowish white, never becoming gray as in the foregoing experiment.

The roots in the solutions of pH 3.0 and pH 3.5 began to decay on the 6th day of the experiment and two days after, those in the solutions of pH 8.0 showed the same result. At the end of the experiment, roots in the solutions more acid than pH 5.6 were somewhat unsound in appearance. It is interesting to see that though the growth of root was equally bad in the extremely acid and extremely alkaline solutions, the growth of the shoot was evidently better in the alkaline solutions. No doubt, the great amount of ammonia absorbed by the seedlings in alkaline solutions was the cause of this superior growth.

Pl. I shows the photograph of the seedlings at the end of this experiment. The dry weight of shoot and root is shown in Table 37a and illustrated in Text-fig. 24. It will be seen that there is no particular maximum in the growth of seedlings in the solutions of varying pH-values. Within the pH-range 4.5–7.0, seedlings could grow very well. Among these, the growth in solutions of pH 5.4 and pH 6.2 was comparatively bad. The growth in the pH-range from 5.8 to 7.0 was the best of all, a result which is in agreement with the findings of earlier works (Loo, 1927 and 1928). It should be noted that the growth in the neutral and alkaline solutions in this experiment was very different from that in the foregoing experiment where we have seen that almost no growth took place in the shoot of seedlings in the alkaline solutions and that generally growth was better in the pH-range 4.6 to 5.8. In the present case, however, the greatest growth was secured in the solutions of pH 6.8 and pH 7.0. Even in the alkaline solutions, the growth of shoot was as good as that in the acid solutions ranging from pH 5,0 to pH 5.4. No wilting of shoot could be observed in this case, though the growth of root was hindered by the extremely alkaline reactions. Thus plant growth in solutions of the same pH-value may be varied according to the ionic condition of the solution. It is almost meaningless to regard the pH-value of a particular solution in which the plant grows most favourably as the optimum pH for the growth of a certain plant. On the whole, however, the growth feature of the root system has a closer relation to the pH-value of the culture solution, and the differences of colour, length, and thickness of the root between acid and alkaline solutions were common to both experiments.

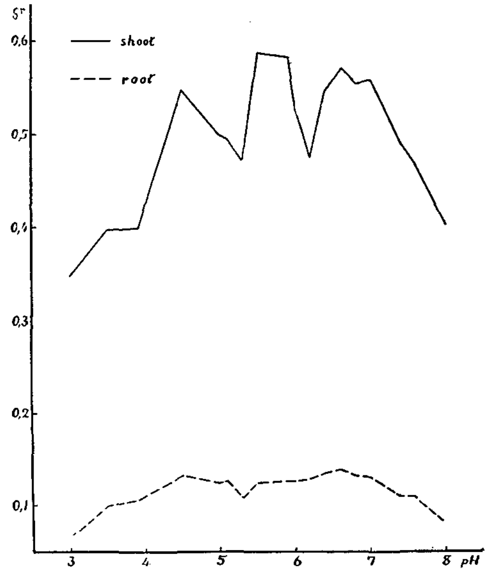


Fig. 24

The amount of ammonia absorbed every day is recorded in Table

37b. Up to the third day, there were points of depression in the curve at pH 5.2, pH 6.4 and pH 8.0 (initial values) (Text-fig. 25). The depression at the initial pH 5.2 shifted to pH 5.4 between the 4th and the last day, partly because the growth of seedlings in this solution was also inferior and partly because the initial pH of this solution always changed to pH 5.2 after the third day. The second depression at the initial pH 6.4 disappeared owing to the superior growth of seedlings in this solution and was replaced by its neighbour pH 6.2–6.1. From Table 37a, we have seen that the growth of shoot in the solution of the initial pH 6.4 was much greater than that in the solution of pH 6.2. It might be expected that the requirement of ammonia must be far greater in the former than in the latter. Consequently this point of depression shifted to pH 6.2–6.1, a pH-value less favourable for the growth of seedlings. In fact, when the total amount of nitrogen absorbed was divided by the dry weight of the root, a curve of three points of depression at pH 5.4–5.2, pH 6.2–6.1 and pH 7.0 was found (Text-fig. 26) just as in the case of the foregoing experiment.

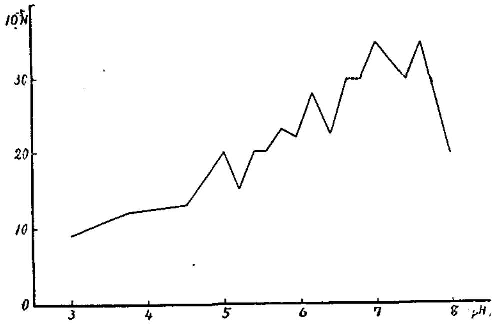


Fig. 25

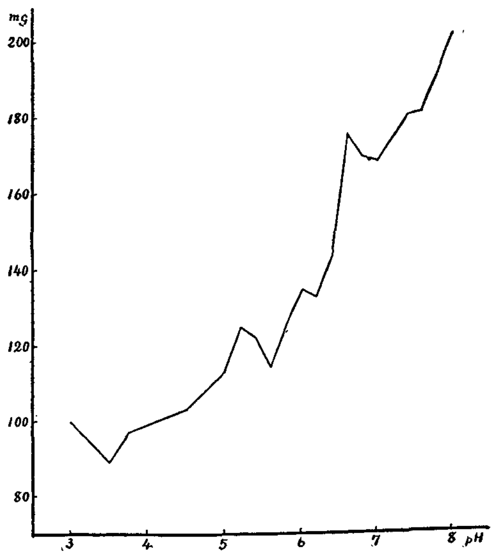


Fig. 26

The absorption was better on the neutral and alkaline side than in the acid solutions. This phenomenon remained unchanged throughout the whole experiment. No decrease of absorption in the alkaline solutions as in the case of the foregoing experiment was recognized in this experiment. No death of shoot occurred in the alkaline solution in this case. Since the amount of ammoniacal nitrogen absorbed was tolerably great on the alkaline side in the foregoing experiment, death of shoot must be caused by hinderance of nitrogen assimilation due to the unfavourable effect of unbalanced solution. When the ammoniacal nitrogen absorbed could be utilized to some extent by the seedlings as in the present case, the shoot of seedlings could grow well in spite of the poor growth of the root system.

The results of experiments with longer duration agree in that the absorption was better in the alkaline than in the acid solutions, that there are definite points of depression in both growth and absorption curves and that the points of depression are almost identical between the two curves. In spite of many variations in the experimental conditions, the amount of ammonia absorbed by the root system in both balanced and unbalanced solutions in evidently less at ca pH 5.2, 6.2–6.4 and 7.9 (sometime at ca pH 7.0) than at the neighbouring pH-values. These points of depression may shift from one place to another either with the change of reaction in the solution or with the effect of growth of seedlings. By daily determination of the pH-value of the culture solutions and by expressing the intensity of absorption in total amount of ammonia absorbed per one gram of dry weight of root, it was found that the results of experiments of longer duration are in general coincident with those of shorter duration.

It is interesting to find that the points of depression in the absorption curves were the same or at least near those in the growth curves. In the experiment with unbalanced solutions, the points of depression for absorption were situated at pH 5.2–5.1, and pH 6.4–6.3, while those of growth were found at pH 5.4–5.2 and pH 6.2. The absorption curve in the case of balanced solution sank at pH 5.6–5.4 and pH 6.2, while in the growth curve it was at pH 5.4–5.2 and pH 6.1. This fact indicates that in these pH-values, the physiological functions of the seedlings, including absorption of nutrient and growth, are reduced to the minimum. In the alkaline solution, however, no such correlation between the absorption and growth could be found; the growth of seedlings declined as the alkalinity of the solution increased.

Another noteworthy point is that with few exceptions a greater amount of ammonia was absorbed by the seedlings which grow most favourably. This result is in agreement with the conclusion of TRUE and BARLETT (1916), who stated that the gaeatest absorption of salts occurs in the most favourable growth. But the acidity or alkalinity where the plants grow most favourably, does not always coincide with that of the most acitve absorption, because the former results and becomes visible long after the latter. But the two cvrves are similar in form with only a few exceptions. On the alkaline side the curve of growth is just opposite to that of absorption; the greater the alkalinity, the greater the absorption of ammonia, but the less the growth. From this fact, ARRHENIUS' statement that at the maximum growth the intake of salt at the minimum (ARRHENIUS, 1922) seems to be true. But from the other facts that a very small amount of nitrate, but a large quantity of ammonia was absorbed in the alkaline solution, it should be concluded that the absorption of ammonia in the alkaline reaction is a special case and that the unfavourable growth in this case was caused by alkalinity directly or indirectly, but has no relation to the absorption.

The results of Experiments 21–38 described above show clearly that the absorption of ammonia and nitrate is strongly affected by the hydrogen ion concentration of the culture solutions. A greater amount of ammonia is absorbed in the alkaline solutions than in the acid ones, but the absorption of nitrate is usually better in weakly acid solutions. This result agrees with the findings of HOAGLAND, THERON, PRIANISCHNIKOW and others and seems to lead to the conclusion that absorption is a process of chemical combination. But if one examines the data more closely, one would hesitate to draw such a conclusion. It is true that the absorption of ammonia is better in alkaline solutions, but this is not the case in the absorption of nitrate. No superior absorption of nitrate took place in the acid solution in the case of decapitation and even in the case of normal plant, slightly superior absorption of nitrate occurred only in the weakly acid solutions. On the contrary, the absorption of nitrate in the extremely acid solutions was usually almost as poor as that on the alkaline side. Moreover, if the absorption of ammonia and nitrate was a phenomenon of chemical combination, it should be expected that the absorption of ammonia would occur in the alkaline, and nitrate in the acid solution. But this is not the case. As the data of the foregoing experiments show, with the exception of alkaline solutions, the absorption of ammonia is always parallel to that of nitrate, and even on the alkaline side, a small amount of nitrate is always absorbed by the root system. From the considerations above mentioned, it is more probable that the absorption of electrolytes by plants is a phenomenon of complicated rather than pure chemical combination. Of course, it does not mean that chemical combination does not take part in the process of absorption. Since protoplasm is an ampholyte or, properly speaking, a complex mixture of amphoteric colloids, it must dissociate in acid or alkaline solutions and consequently it might be expected that chemical combination may occur at the very first moment when the protoplasm comes into contact with electrolytes. But absorption is a process which involves the penetration of substance through the protoplasmic layer into the cell sap; mere chemical combination cannot account for the whole. Regarding this connection, recent researches of WL. S. BUTKEWITSCH and W. W. BUTKEWITSCH (1929) are interesting. These authors worked with a collodium sack. When the sack was filled with distilled water and immersed in the ammonium nitrate solution of different pH-values (by adding HCl or NaOH), it was found that the diffusion of ammonia into the collodium sack was greater in the alkaline than in the acid solution. But as for the diffusion of nitrate, it increased with the increase of acidity. Similar result was obtained by the same workers in the diffusion of phosphate using the same method. Thus it is clear that diffusion is strongly affected by the hydrogen ion concentration of the solution. It also becomes evident that comparatively superior absorption of cations in the alkaline and of anions in the acid solution does not necessarily mean the possibility of chemical combination.

When the amount of ammonia or nitrate absorbed is plotted against pH, instead of a curve with one maximum, cvrves of wave-shape are obtained in which more than three points of depression are found. These points are situated on the curve respectively at pH 5.1–5.4 (usually at pH 5.2), pH 6.1–6.4 (usually at pH 6.4), pH 7.0–7.2 and pH 7.8–7.9. The latter two points of depression are not constant; sometimes one disappears, sometimes the other. This phenomenon is found in the experiments with different kinds of buffer mixture and culture solutions having uniform amount of either cations or anions. Thus the maxima and minima were expressed schematically as follows:

3.0 / 4.5 \ 5.2–5.4 / 5.8–6.0 \ 6.2–6.4 / 6.8 \ 7.0–7.2 / 7.6 \ 7.8

This phenomenon can be easily understood by assuming the protoplasm as a polyphase system of amphoteric colloids, each with an isoelectric point. From the chemical point of view, the isoelectric point of an ampholyte can be expressed by hydrogen concentration in which it exists practically in minimum dissociated condition. In this condition, the sum of anions and cations of the ampholyte is the minimum and the concentration of anions is equal to that of cations (LOEB, 1922, p. 10; MICHAELIS, 1922, S. 57). That is, on the acid side of the isoelectric point, the ampholyte behaves like a base, and is capable of forming salts with anions; on the alkaline side of this point, it behaves as an acid, capable of combining with cations; but just at the isoelectric point, the power of chemical combination is at the minimum, it can hardly form salts with cations nor with anions. On the other hand, from physical points of view, the physical properties of the protoplasm have been found to be smallest at the isoelectric point; that is to say, besides the loss of electric charge, the degree of viscosity and swelling of protoplasm is so low at the isoelectric point that it is unfavourable for the penetration of salts and other substances. At any rate, the physiological function of the protoplasm should be expected to be minimum at the isoelectric points. In the case of absorption of ammonia and nitrate by root system, if there are many isoelectric points in the protoplasm of the root tissues, one would expect that the depression of absorption has to do with these points and hence the wave-formed curves. This relation holds good with the growth of seedlings too, when they are grown in the solutions of different pH-values.

The wave-formed curves with more than one maxima were usually observed in the studies on the influence of hydrogen ion concentration upon the physiological functions of plants, such as germination of seeds, germination of fungus spores, growth of seedlings, absorption of water and salts, penetration of dyes etc. WEBB (1919, 1921) found depression in the percentage of germination of spores at pH 7.0 in the cases of *Aspergillus niger* and *Penicillium cyclopium*, and at pH 6.2 in the case of *Fusarium* species. He also found that the point of depression varied to some extent under different temperature. For example, in the case of *Penicillium cyclopium* the minimum point of germination shifted to pH 6.2 when the experiment was carried out under 27°C. SALTER and MACILVAINE (1920) performed a series of experiments with regard to the influence of hydrogen ion concentration on the germination and growth of a number of plants. From their results, it will be seen there are some minimum points for germination of seeds in the cases of *Triticum vulgare*, *Trifolium pratense*, *Glycine hispida* and *Medicago sativa*. HIXON (1920) found that the ash content of root system of wheat plants grown in solutions of different pH-values has a minimum point at pH 6.0. He also found a mimimum at pH 5.0 for the germination of seeds of *Pisum sativum*. HIXON was the first who regarded the point of depression between two maxima as the isoelectric point of the protoplasm. E. F. HOPKINS (1921, 1922) found depression in the growth of *Gibbella saubinetii* and of its ability to infect wheat seedlings at pH 5.0. Similar results were obtained by LUNDEGÅRDH (1924a) in the case of *Gibberella saubinetii* and *Fusarium* sp., and by LINDFORS (1924) in the case of *Fusarium minimum*. HERCIK (1925) found two maxima for the growth of roots of *Pharibitis hispida*, one at pH 5.8, the other at pH 7.5. From 1922 to 1925, ARRHENIUS carried on an extensive study on the influence of hydrogen ion concentration of the soil upon the growth of several cultivated plants. A majority of growth curves plotted against pH has two maxima and one minimum between them. D. L. HOPKINS (1926) found a curve of two maxima in the growth, division and movement of *Amœba proteus*. ILJIN (1928) found a point of depression in the excretion of sugar and KCl from plant tissue when the amount of substances excreted was plotted against pH. Most recently BERG (1929) found that curves for germination of pollen and growth of the pollen tube in sugar solutions of varied reaction have two maximum points. Not all the authors cited above considered the minmum as the isoelectric points of the protoplasm. Indeed, the final reaction of culture medium, especially in the case of fungi, usually changed to such an extent from the initial that it is not altogether reasonable to regard the initial pH-value at which the growth was depressed as the isoelectric point of the protoplasm. But it is not wholly absurd to consider the curve of maxima as a phenomenon which must be at least correlated to some extent with the amphoteric property of the protoplasm.

ROBBINS and his school studied the problem of isoelectric point of the protoplasm. He was able to show that when potato tuber tissue was immersed in the dilute buffer solutions of different pH-values, the absorption of water is less at pH 6.0 than at the neighbouring pH-values. The absorption and retention of acid dyes are more strong on the acid side of pH 6.0, and of basic dyes on the alkaline side of that point. It shifts the reaction of the dilute buffer solutions towards an equilibrium point near pH 6.0 (ROBBINS, 1923). Thus potato tuber tissue responds like an ampholyte with an isoelectric point near pH 6.0. The same result was obtained by ROBBINS in the experiments with the mycelium of *Rhizopus nigricans* and *Fusarium lycopersici* (1924). They absorbed and retained the acid and basic dyes as if they had isoelectric points in the vicinity of pH 5.0 and pH 5.5 respectively. By using MICHAELIS' method of determining the isoelectric point, SCOTT, (1924) and ROBBINS and SCOTT (1925) secured pH-values for each kind of material employed at which the reaction of dilute buffer solutions comes to an equilibrium, namely: for potato tuber tissue, pH 6.0; for root tip of soybeans, pH 6.2–6.4; for mycelium mats of *Fusarium lycopersici*, pH 5.5 ; for the same of *Gibberella saubinetii*, pH 6.4 ; for *Fusarium oxysporum*, pH 4.9. In a later paper (1926), ROBBINS found characteristic points situated at pH 6.0–6.4 in absorption of basic and acid dyes, in the absorption of water in solutions of toxic cations and toxic anions by potato tissues, and in the change of ash content of potato tissue in dilute buffer mixtures. In the case of *Elodea*, he recognized similar phenomenon in the influence of hydorogen ion concentration on the toxicity of acid and basic dyes and the change of reaction of dilute sodium phosphate buffer mixtures. In these buffer solutions, acid dyes are more toxic to *Elodea* in solutions more acid than pH 6.0 or pH 6.2 than in the less acid and the more alkaline solutions. On the other hand the basic dyes are more toxic in solutions less acid and more alkaline than pH 6.0–6.2 than in solutions of higher acidity. When a piece of *Elodea* was placed in the dilute buffer solutions of various pH-values, the reaction of the solution changed continuously till it reached a point of equilibrium at pH 5.85–6.68. Thus the results of ROBBINS and his coworker show clearly that in the solutions of different pH-values, plant tissue acts as if it were an ampholyte with a characteristic point which may be regarded as the isoelectric point of the protoplasm.

On the other hand, COHN, GROSS and JOHNSON (1921) determined the isoelectric point of the pressed juice of potato, carrot and tomato by the method of cataphoresis and found that the isoelectric point of tuberin, the chief protein of potato tissue, is about pH 4.0. Similar results were secured by PEARSALL and EWING by the precipitation method. It is noteworthy that the isoelectric points of plant juices determined by COHN, GROSS and JOHNSON and PEARSALL and EWING (1924a) are perfectly or almost coincident with those of the chief protein of these plants.

It is worthy to note, however, that what ROBBINS found to be the isoelectric point for potato tissue is quite different from that of tuberin found by COHN, GROSS and JOHNSON. SCOTT (1929) is of the opinion that potato tissue is composed of more than one protein, besides tuberin ; there are other ampholytes each with its own isoelectric point ; the isoelectric point of potato tuber tissue is noting but the resultant of the isoelectric points of these ampholytes.

YOUDEN and DENNY (1926, 1927) made objections to the assumption of isoelectric points, which were found by ROBBINS and his co-worker by their method. YOUDEN and DENNY immersed the tissue of apple, potato, and barley roots which had been cut to pieces, seeds of corn, rye and wheat and corn powders in a series of buffer and non-buffer solutions and found that the pH equilibrium reached in these solutions is the same as that obtained by placing the tissue in distilled water. The change of reaction of buffer solutions is believed to be due to substances leaching out of the tissue, substnaces which were not coagulated by heat or soluble in acid alcohol. They also found that the dialyzable materials of plant tissues had a greater buffer effect than the non-dialyzable ones. These facts show that proteins or other colloidal substances in the protoplasm do not play an important rôle in changing the reaction of buffer solutions. Thus YOUDEN and DENNY were inclined to deny the protein anology claimed by ROBBINS and his co-worker. It is a matter of doubt, however, whether YOUDEN and DENNY's results are sound enough to explode ROBBINS' hypothesis. Their results only show the fact that something leaches out of plant tissue which has a regulating effect on the pH of buffer and non-buffer solutions. This is all and nothing more. The writer has found that almost all inorganic elements were excreted by the turnip tissues when they were washed several times with distilled water and placed in the dilute solutions of ammonium nitrate. It is therefore possible that there may be some defect in the method of experimentation in some of ROBBINS' works when he determined the isoelectric point of plant tissue by placing them in the buffer solution in order to find the pH-equilibrium. But ROBBINS' conclusion is based on the results obtained by several methods such as the absorption and retention of dyes, absorption of water, change of ash content etc. There is no justification for YOUDEN and DENNY to doubt the protien analogy, which was claimed by ROBBINS, when they found that someting leaching out of the tissue affected the reaction of buffer solution. In fact, SCOTT (1929) recently showed that what is claimed by YOUDEN and DENNY is not true if the materials used are living and have been washed thoroughly with distilled water before immersing them into the buffer solution.

While ROBBENS' hypothesis is elaborated from the purely chemical standpoint, PEARSALL and EWING have worked on the existence of isoelectric point in the protoplasm with methods considering the physical properties of the protoplasm. These English investigators performed experiments with several kinds of plants on the influence of hydrogen ion concentration upon the exosmosis of Cl and anthocyan from the plant cell. They regarded the pH-value at which the exosmosis of Cl and anthocyan pigments took place as the isoelectric point of the protoplasm of plant in question. By this method, they found the isoelectric point for potato to be pH 4.4; for sugar beet, ca. pH 4.3; for carrot, pH 4.25; for *Nitella*, ca. pH 4.4 (1924a). PEARSALL and EWING (1927) found a minimum point (minimum of swelling) in the increase of volume and weight resulting from the absorption of water by the tissues of *Solanum tuberosum*, *Brassica Napus* var. *Napobrassica*, *Brassica Rapa*, and *Vicia Faba* which were immersed in the buffer solutions of different pH-values. These minimum points were as follows:

Tissues of plants	isoelectric point
Solanum tuberosum	pH 3.2
Brassica Napus var. *Napobrassica*	„ 4.5
Br. Rapa	„ 5.2–5.4
Vicia Faba	„ 6.2–6.5

They explained the cause of these phenomena on the change of permeability of plasma-membrane. According to them, the plasma-membranes lose their semi-permeability in this specific pH so that the capacity of salt and water retention is lost. It seems to the authors that the loss of the semi-permeability is due to the precipitation or coagulation of plasma-colloid.

Besides the works cited above which indicate the existence of the isoelectric point of the protoplasm, many authors have found similar phemena: (1) in absorption (DAVIDSON, 1927; SABININ and KOLOTOVA, 1927; NIKLEWSKI, KRAUSE and LEMANCZYK, 1928) and excretion of salts (OSSIPOWA and JUFEREWA, 1926); (2) in the production of pH-equilibrium in buffer solutions by seeds (RUDOLF, 1922, 1925) and succulent tissue (ULEHLA, 1928); (3) in the growth of root hairs (FAR, 1927); (4) in the relation of the toxic effect of cations and anions (SCOTT, 1929) and (5) in the antagonistic action of salts (MEVIUS, 1927). These results show that protoplasm behaves like an ampholyte with a characteristic point which seems to have some relation to the isoelectric point of the protoplasm.

It should be pointed out, however, that with a few exceptions, all authors cited above, regard the protoplasm as a simple ampholyte with only one isoelectric point. That protoplasm is a polyphase system which consists of many amphoteric substances such as proteins, amino acids, lipoids etc. is a fact out of question. These amphoteric substances form a colloidal mixture in the cell, but do not always take the form of a chemical compound. Consequently, if there is any possibility of the existence of an isoelectric point in the protoplasm, instead of a single one, more than one point should be expected. In fact, MCDOUGAL and MORAVEK (1927) found that in a constructed cell with colesterine and lethicin there was a point of depression in exosmosis of ions when the cell was immersed in the solutions of different pH-values. Their results show that not only proteins but also lipoids are influenced by the hydrogen ion concentration. In ROB-

BINS' later work (1926), there are many curves of polymaxima of which he has taken no account. Such curves could not be satisfactorily explained on the assumption of only one isoelectric point. Our figures all show that the chief protein as well as other amphoteric substances of the protoplasm play rôle in showing a wave-formed curve in relation to the absorption.

On the contrary, STEARN (1926 a & b), from both theoretical and experimental results, concluded that in a complex system containing more than one colloid each with its own isoelectric point, there is only one isoelectric point which is different from those of both the component colloids. This fact seems to serve as a basis for the truth of ROBBINS' and other's hypothesis. But one must not be too rash to anticipate the analogy between test tube and plant cell. The colloidal mixture in a living plant cell is quite a different thing from that in the test tube. The protoplasm of a living organism is a complex system of various chemical natures and in various states of aggregation (BAYLISS, 1920, p.26) and might be expected to react individually.

The hypothesis of the existence of more than two isoelectric points in the protoplasm was first advanced by SAKAMURA and LOO (1925) based on the results of a series of experiments which were performed with regard to the influence of hydrogen ion concentration upon the softening and hardening of protoplasm of *Spirogyra*. After immersing the filaments of *Spirogyra* in the dilute buffer solution ranging from pH 3.5 to pH 8.2 for 5 minutes, they were centrifuged and the degree of displacement of chloroplasts from cytoplasmic layer was observed. The degree of displacement of chloroplasts was divided into four different states, the most displaceable state was considered as due to the softening and the contrary as due to the hardening of protoplasm by the influence of hydrogen ion concentration. By such methods, SAKAMURA and LOO obtained curves of three maxima and three minima when the percentage of filaments which were softened or hardened by hydrogen ion concentration of the solutions was plotted against pH. Though the points of maxima and minima were variable to certain degree according to the seasons in which the materials were collected and other external conditions such as temperature, change of reaction in the solution during experiment etc., they were in general situated in the vicinity of the following pH-values.

For plants in the winter and spring

softening	4.7		6.0		6.8	
hardening		4.9		6.4		7.5

For plants in the summer

softening	4.7		5.8		7.2	
hardening		5.3		6.4		7.6

This phenomenon was explained by considering the protoplasm of *Spirogyra* as a mixture of more than two amphoteric colloids which, respectively posses isoelectric points. According to SAKAMURA and LOO, the wave-shaped curves are caused by "ein Resultant der mehreren Kurven der Zustandsänderungen einiger Plasmabestandteile ist. Die Verfestigungspunkte dürften die isoelektrischen Punkte darstellen, wo der Verfestigungsgrad aber durch die gegenseitige Wirkung von anderen kolloidalen Bestandteilen etwas modifiziert wird."

To this hypothesis of polyisoelectric points of protoplasm as advanced by SAKAMURA and LOO, many authors express opinion of approbation (for example, GICKLHORN, 1927; PFEIFFER, 1928). STRUGGER (1926) studied the influence of hydrogen ion concentration of solutions upon some morphological properties of protoplasm and the rate of protoplasmic streaming in the root hairs of *Hordeum vulgare* under the dark ground illumination. Within the pH-range of 5.9 to 6.8, he obtained curves of the two maxima for both cases. A little later (1928), the same author continuing his studies with the same material and method, again found curves of three maxima between pH 6.8 and pH 7.5. In theoretical consideration, STRUGGER adopted the hypothesis of SAKAMURA and LOO in the explanation of the phenomena. It should be noted, however, that there are too many successions of maxima and minima in STRUGGER'S curves within such a small pH-range. These points of minima may have some relation to the isoelectric points of the protoplasm, but can not be regarded as isoelectric points themselves.

On the other hand, HEILBRUNN (1928, pp. 188–192) attempted to find faults in SAKAMURA and LOO'S work. His criticism consists in two points. Firstly he collected the data of all experiments into two tables and found that they are not exactly consistent. For example, he averaged the percentage of displacement of chlorophyll-filament of all experiments at pH 5.2 and neighbourhood and concluded that there is no "maximum of viscosity" (p. 192). Here it should be noted that the data in each table of SAKAMURA and LOO'S work are the results of different experiments, where slite variation of pH-point of hardening and softening can not be avoidable. How could one average the percentage of displacements which were determined under such a condition? The second point is that some pH-values which "SAKAMURA and LOO state to be" (according to HEILBRUNN) the points of hardening or softening have no record in the data. SAKAMUKA and LOO did not state which pH-value or values were the point or points of softening and hardening; they only stated that such points lie in the vicinity of these pH-values. On the whole, HEILBRUNN'S criticism on SAKAMURA and LOO's work is based on misunderstandings.

The results presented in this part of the present work furnish other data which support SAKAMURA and LOO'S statements. It is very interesting to see that the curve of absorption of ammonia and nitrate is very much like that of the change of physical properties of protoplasm in the case of *Spirogyra*. We have seen that in our case of absorption, too, the form of the curve varied to some extent under different conditions. But notwithstanding these unavoidable variations, the maxima and minima in the curves are situated in a definite pH-zone which is so evident that one can not fail to explain the phonomenon on the ground of the existence of isoelectric points in the protoplasm.

The next question is: Do all these points of maxima or minima mean isoelectric points? The position of maximum or minimum points may be different from the real isoelectric points of the protoplasm, but no doubt it lies near the vicinity of them. In a recent article, PFEIFFER (1929, S. 1563) hesitates in admitting the existence of many isoelectric points in the same tissue, because the polymaxima of the curves can often be reduced by the addition of certain salts to the solution. Unless there is some evidence of production of polymaxima in the curve resulting from the addition of salt, it is not very legitimate to doubt the existence of many isoelectric points in the protoplasm. For the reduction of polymaxima in the curve might be the influence of the salt, but it does not follow that the maxima or minima may be increased by the addition of salts. Our data described in the above show that when the reaction of the buffer solution was changed seriously on the alkaline side, the depressions on that side may be unrecognizable. However, there was no single evidence that these maximum or minimum points have been increased by the effect of a certain salt, though different kinds of buffer solutions were employed in the experiments.

Now rises the question: Which represents the isoelectric points, the maxima or the minima? As has been pointed out, from both physical and chemical standpoints, the minimum points should be regarded as to do with isoelectric points. PFEIFFER (1929) and GELLHORN (1929) are of the same opinion. On the other hand, authors like LLOYD (1915), FREE (1916) HAYNES (1921) and PEARSALL and EWING (1924b, 1925, 1927) are inclined to consider the permeability of the protoplasm to be at its maximum at the isoelectric point. According to LLOYD, FREE and HAYNES, protoplasm consists of a hydrophile colloid, its permeability is determind by the change of water content of the disperse phase; the permeability will be lessened through swelling of the disperse phase, because the diffusion way of the protoplasm may become narrow through the swelling. Therefore at the isoelectoric point, the permeability will be the greatest, since at that point the swelling of the disperse phase is at its minimum. PEARSALL and EWING are of the opinion that at the isoelectoric point precipitation or coagulation of plasma-colloid takes place so that the protoplasm loses its semipermeability. All these authors agree with each other in that the swelling of the disperse phase of plasma-colloid is least at the isoelectric point. As has been pointed out by GELLHORN (1929) and PFEIFFER (1929), from the fact that the electrolytes which accelerate the swelling of the disperse phase of colloids increase the permeability, while those reduce the swelling, hinder the permeability, it is more legitimate to expect that the permeability would be least at the isoelectric point. Fortunately the recent work by RISSE (1926 a & b) show that is actually the case. RISSE found that the permeability of a gelatine or a collodium membrane to water and the dissolved substances was increased by the increase of swelling of the membrane and that at the isoelectric point, the permeability is least. Thus it is beyond question that in our case in the root tissue of maize the points of depressions mean the isoelectric points of the protoplasm or the resultant of the isoelectric phenomena.

In conclusion, the absorption of ammonia and nitrate by the root system of *Zea Mays* is strongly affected by the hydrogen ion concentration of the external solutions. The cause of this influence lies in the change of permeability of the root cell due to the change of physico-chemical properties of the plasma-colloids and this seems to have some relation to isoelectric points of the protoplasm.

The general discussion on the absorption of salts by the root system of the higher plants will be reserved for another occasion of the publication of the results of the further related experiments, which were already carried out.

Summary

1. The reaction of culture solution containing ammonium nitrate changes rapidly in contact with the root system of *Zea Mays*. This reaction change, in turn, has effect upon the growth of seedlings. The unequal absorption of ammonia and nitrate from ammonium nitrate which is the chief cause of the change of reaction, is strongly affected (a) by the concentration of the nutrient solution, (b) by hydrogen ion concentration of the solution, (c) by the presence of other salt or ion in the solution and (d) by the growth of seedlings and demand of nutrients in the part of seedlings.

2. If the concentration of the nutrient solutions is relatively high, ammonia is absorbed at first and nitrate is little or not at all absorbed by the seedlings of *Zea Mays*. Until ammonia is absorbed to a certain extent, the absorption of nitrate does not begin. On the other hand, if the concentration of the culture solution is low, nitrate is absobed at the very beginning of the experiment. The degree of reaction change caused by unequal absorption of ammonia and nitrate depends in the main upon the difference of amount of ammonia and nitrate absorbed.

3. If the concentration of culture solution is very high, a much larger amount of ammonia than nitrate is absorbed by the seedlings, the acidity of the solution increases rapidly and considerably so that the growth of the seedlings in reamarkably hindered, In the serious cases, the seedlings died from the extreme acidity. If the concentration of culture solution is low, nitrate is absorbed as well as ammonia, therefore the increase of acidity is not so rapid and great as in the case of concentrated solutions.

4. If the concentration of ammonium nitrate is remarkably less than other salts in the solution, nitrate is better absorbed than ammonia. In this case, the change of reaction in the solution is not governed by the difference in absorption of ammonia and nitrate, the solution becomes more and more acid as the experiment proceeds. The absorption of ammonia and nitrate from a pure solution of ammonium nitrate is similar to that from a complete solution.

5. When the seedlings of *Zea Mays* are grown in the nutrient solution of different reactions, the amount of ammonia absorbed increases with the decrease of acidity and increase of alkalinity of the culture solution. Nitrate, however, is relatively better absorbed at a weakly acid reaction. But even in weakly acid solutions, there is sometimes evidence of a superior absorption of nitrate to that of ammonia. The absorption curve is wave-formed having more than three points of depressions. These points are respectively

situated on the curve at pH 5.1-5.4 (usually at pH 5.2), pH 6.1-6.4 (usually at pH 6.4) pH 7.0-7.2 and pH 7.8-7.9.

6. This phenomenon is explained by assuming that the protoplasm is a mixture of amphoteric colloids each with its own independent isoelectric point. At these isoelectric points, the swelling of plasma colloid is at the minimum, hence the depression of absorption curve may have some connection with these points.

7. When the yield expressed in dry weight of seedlings grown in nutrient solutions of various reactions which are renewed every 24 hours is plotted against pH, a wave-like growth curve is obtained with many maxima and minima. The positions of the maxima and minima in this curve are coincident with those of the absorption curve or at least lie in the vicinity of them. In the case of extremely alkaline solutions, however, the growth curve shows just the opposite relation to the absorption curve. The amount of ammonia absorbed increases with the increase of alkalinity in the solution, but the yield in dry weight is inversely proportional to the alkalinity. In the solutions of the same reaction, the absorption of nitrogen and growth of seedlings are different, according to the difference in concentration of salts used as buffer and in ionic conditions (physiologically balanced or unbalanced) of the nutrient solutions.

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VI. Exprimental data

Table 1

Zea Mays: 3 seedlings grown in 350 cc. of complete solution which contained 0.02400 N. ammonium nitrate. Initial pH=4.6. Experimental duration: Jan. 22–Feb. 5, 1927. Temp. 20–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]-[MO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	4	3.1	177	0	177	0.1758	49.25	0	
2	9	3.0	347	106	241	0.1918	88.74	29.09	3.27
3	14	3.0	351	100	251	0.2192	78.54	22.35	3.51

Table 2

Zea Mays: 3 seedlings grown in 350 cc. of complete solution which contained 0.01215 N. ammonium nitrate. Initial pH=5.0. Experimental duration: Jan. 12–27, 1927. Temp. 20–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]-[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.8	55	0	55	0.0953	28.29	0	
2	3	3.4	103	42	61	0.1110	45.42	18.54	2.45
3	5	3.4	137	42	95	0.1187	57.00	17.34	3.26
4	7	3.4	135	44	91	0.1092	60.57	19.74	3.07
5	15	2.7	465	245	220	0.2795	81.60	42.90	1.90

Table 3

Zea Mays: 3 seedlings grown in 300 cc. of complete solution which contained 0.00354 N. ammonium nitrate. Initial pH=4.8. Experimental duration: Feb. 9-28, 1927. Temp. 18–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]$-$[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (gm.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.8	38	2	36	0.1413	11.31	0.59	19.00
2	3	3.2	113	52	61	0.1905	24.90	11.46	2.17
3	5	3.0	179	107	72	0.1817	41.40	24.75	1.68
4	6	3.0	250	154	96	0.2282	45.30	28.02	1.62
5	7	3.0	296	180	116	0.2407	51.60	31.41	1.64
6	8	2.9	332	186	146	0.2638	52.80	29.64	1.79
7	10	3.0	346	201	145	0.2873	50.55	29.40	1.72
8	12	3.5	353	329	24	0.2372	62.46	58.20	1.07
9	13	3.5	354	335	19	0.3103	47.88	45.30	1.06
10	15	4.2	354	354	0	0.3615	41.10	41.10	1.00
11	19	4.8	354	354	0	0.3238	45.90	45.90	1.00

Table 4

Zea Mays: 3 seedlings grown in 350 cc. of complete solution which contained 0.00122 N. ammonium nitrate. Initial pH=5.4. Experimental duration: Jan. 12-24, 1927. Temp. 20-25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]$-$[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	4.0	47	40	7	0.1045	22.05	18.69	1.18
2	3	3.6	49	42	7	0.0820	29.25	25.11	1.17
3	5	3.2	88	62	26	0.1083	39.78	28.05	1.42
4	7	3.1	103	66	37	0.1105	45.60	29.04	1.56
5	10	3.0	121	87	34	0.1183	50.10	38.10	1.39
6	12	3.5	121	100	22	0.1270	46.80	38.58	1.21

Tabls 5

Zea Mays: 3 seedlings grown in 400 cc. of complete solution which contained 0.01192 N. ammonium nitrate. Initial pH=4.8. Experimental duration: Feb. 10-23, 1927. Temp. 18-25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]$-$[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.6	86	2	84	0.1295	37.15	0.86	43.00
2	4	3.3	117	36	81	0.1415	46.30	14.25	3.25
3	6	3.0	217	72	145	0.1560	77.85	25.80	3.01
4	8	2.9	312	102	210	0.1835	95.25	31.50	3.06
5	9	2.8	402	199	203	0.2101	107.10	53.00	2.02
6	11	2.7	542	254	288	0.2214	137.25	64.50	2.13
7	13	2.7	482	259	233	0.1861	145.30	78.25	1.86

Table 6

Zea Mays: 3 seedlings grown in complete solution of 400 cc. which contained 0.00593 N. ammonium nitrate. Initial pH=4.8. Experimental duration: Feb. 10–23, 1927. Temp. 18–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]$-$[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.8	34	7	27	0.1302	14.57	3.01	4.86
2	4	3.3	121	37	84	0.1485	45.65	13.97	3.27
3	6	3.0	233	100	133	0.1556	83.85	36.00	2.33
4	8	3.0	278	126	152	0.1686	92.50	41.85	2.21
5	9	3.0	268	118	150	0.1614	93.00	40.80	2.28
6	11	2.9	429	224	205	0.2406	100.00	52.15	1.92
7	13	2.7	540	325	215	0.2360	128.15	77.35	1.66

Table 7

Zea Mays: 3 seedlings grown in complete solution of 400 cc. which contained 0.00420 N. ammonium nitrate. Initial pH=4.7. Experimental duration: Mar. 14–23, 1927. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH^{\cdot}_4]$-$[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.7	52	54	−2	0.1542	18.88	19.70	0.96
2	2	3.3	96	100	−4	0.1470	36.58	38.10	0.96
3	3	3.2	110	126	−16	0.1750	35.20	40.50	0.87
4	4	3.0	170	212	−42	0.1800	52.85	66.20	0.80
5	5	3.0	198	216	−18	0.1760	63.00	68.50	0.92
6	7	2.9	284	253	30	0.1875	84.75	75.60	1.12
7	8	2.9	340	301	39	0.2017	94.50	83.50	1.13
8	9	2.8	368	290	78	0.2380	86.55	68.20	1.27

Table 8

Zea Mays: 3 seedlings in each culture containing 400 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00242 N. Initial pH=4.8. Experimental duration: Feb. 26–Mar. 10, 1927. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH'_4]-[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.5	58	66	-8	0.1262	25.75	29.26	0.88
2	3	3.4	107	135	-28	0.1350	44.40	56.20	0.79
3	4	3.3	119	151	-32	0.1362	48.85	61.85	0.79
4	5	3.2	146	186	-40	0.1448	56.45	71.40	0.79
5	6	3.0	210	192	18	0.1716	68.50	62.75	1.09
6	7	3.0	239	206	33	0.1895	70.60	60.85	1.16
7	9	3.0	241			0.2002	67.50		
8	10	3.0	241	231	10	0.1945	69.50	66.75	1.04
9	12	3.0	242	242	0	0.2625	51.65	51.65	1.00

Table 9

Zea Mays: 3 seedlings in each culture containing 400 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00242 N. Initial pH=4.7. Experimental duration; Mar. 14–22, 1927. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH'_4]-[NO'_3]$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.6	56	82	-26	0.1695	18.50	27.20	0.68
2	3	3.2	103	142	-39	0.1440	40.00	54.80	0.73
3	4	3.0	156	142	14	0.1800	48.50	44.20	1.10
4	5	3.0	202	185	17	0.2360	47.98	44.00	1.09
5	7	3.0	241	204	37	0.2355	57.30	48.20	1.19
6	8	3.0	242	242	0	0.2570	52.75	52.75	1.00

Table 10

Zea Mays: 3 seedlings in each culture containing 400 cc. of nutrient solution, Concentration of ammonium nitrate: 0.00121 N. Initial pH= 4.8. Experimental duration: Feb. 26–Mar. 8, 1927. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.6	66	81	-15	0.1488	24.85	30.30	0.82
2	3	3.3	105	116	-11	0.1740	33.80	37.58	0.90
3	4	3.0	119	120	-1	0.1860	35.80	36.10	0.99
4	5	3.0	121	121	0	0.2000	33.90	33.90	1.00
5	6	3.0	121	121	0	0.2000	33.90	33.90	1.00
6	7	3.0	121	121	0	0.2283	29.60	29.60	1.00
7	9	3.0	121	121	0	0.2300	29.45	29.45	1.00
8	10	3.0	121	121	0	0.2300	29.45	29.45	1.00

Table 11

Zea Mays: 3 seedlings in each culture containing 350 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00122 N. Initial pH= 4.8. Experimental duration: June 30–July 6, 1927. Temp. 15–28°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NH_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.5	62	72	-10	0.1450	20.97	24.35	0.86
2	2	3.4	74	83	-9	0.1530	23.70	26.60	0.89
3	4	3.0	119	120	-1	0.1650	35.35	35.70	0.99
4	4	3.0	120	119	1	0.1645	35.68	35.40	1.01
5	6	2.9	121	122	-1	0.1738	34.10	34.43	0.99
6	6	3.0	122	122	0	0.1795	33.40	33.40	1.00

Table 12

Z a Mays: 3 seedlings in each culture containing 350 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00354 N, the amount of salts other than N-source was twice as great as that of KNOP solution. Initial pH=4.6. Experimental duration: Feb. 1–13, 1928. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$	dry weight of the root	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3	(0.00001 N)	(gm.)	NH_3-N	NO_3-N	
1	1	3.8	20	27	-7	0.1190	8.24	11.13	0.74
2	3	3.4	62	74	-12	0.1530	19.85	23.70	0.84
3	6	3.3	104	134	-30	0.1640	31.25	39.85	0.78
4	8	3.0	161	174	-13	0.1850	42.65	45.85	0.93
5	10	2.9	217	208	9	0.1880	56.60	54.30	1.04
6	12	2.9	232	192	40	0.1820	62.50	52.00	1.21

Table 13

Zea Mays: 3 seedlings in each culture containing 350 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00354 N, the amount of salts other than N-source was equal to that of KNOP solution. Initial pH=4.6. Experimental duration: Feb. 1–13, 1928. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$	dry weight of the root	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3	(0.00001 N)	(gm.)	NH_3-N	NO_3-N	
1	1	3.8	26	26	0	0.1285	9.93	9.93	1.00
2	3	3.4	64	67	-3	0.1290	24.30	25.85	0.94
3	6	3.0	134	105	29	0.1410	47.00	36.50	1.27
4	8	3.0	175	177	-2	0.1810	47.40	47.85	0.99
5	10	2.8	255	217	38	0.2054	60.85	52.00	1.17
6	12	2.9	218	234	-16	0.2030	52.70	56.65	0.93

Table 14

Zea Mays: 3 seedlings in each culture containing 400 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00354 N, the amount of salts other than N-source was 5/6 of that of KNOP solution. Initial pH= 4.6. Experimental duration: Mar. 19–31, 1927. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	2	3.2	106	91	15	0.1825	32.50	27.80	1.17
2	3	3.2	126	110	16	0.1700	41.50	36.10	1.15
3	4	3.0	170	130	40	0.1880	50.60	38.60	1.31
4	6	2.9	298	224	74	0.2060	81.00	60.85	1.33
5	9	3.0	345	325	20	0.2270	85.00	77.30	1.06
6	12	3.1	349	340	9	0.2630	74.40	72.20	1.03

Table 15

Zea Mays: 3 seedlings in each culture containing 350 cc. of nutrient solution. Concentration of ammonium nitrate: 0.00354 N, the amount of salts other than N-source was half of that of KNOP solution. Initial pH= 4.8. Experimental duration: Feb. 1–13, 1928. Temp. 15–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absoobed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	4.0	42	11	31	0.1130	18.20	4.77	3.82
2	3	3.4	74	37	37	0.1315	27.55	13.77	2.00
3	6	3.2	94	72	22	0.1430	32.20	24.80	1.30
4	8	2.9	167	154	13	0.1660	49.30	45.70	1.08
5	10	2.9	220	177	43	0.1510	71.45	57.50	1.24
6	12	2.9	206	158	48	0.1560	64.75	49.75	1.30

Table 16

Zea Mays: 3 seedlings in each culture containing 350. cc. of ammonium nitrate solution whose concentration was 0.00483 N. Initial pH=5.4. Experimental duration: June 13–26, 1927. Temp. 10–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.8	47	3	44	0.1480	15.58	0.99	15.66
2	3	3.1	137	83	54	0.1500	44.75	27.10	1.65
3	5	2.8	183	103	80	0.1590	56.45	31.70	1.78
4	7	2.8	223	111	112	0.1560	70.10	34.85	2.01
5	8	2.8	233	113	120	0.1655	69.00	35.50	2.06
6	12	2.7	308	183	125	0.1980	76.25	45.40	1.68

Table 17

Zea Mays: 3 seedlings in each culture containing 350 cc. of ammonium nitrate solution whose concentration was 0.00354 N. Initial pH=5.4. Experimental duration: June 13–26, 1927. Temp. 10–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.7	50	17	33	0.1485	16.50	5.61	2.94
2	3	3.0	120	87	33	0.1515	38.80	23.15	1.38
3	4	3.0	154	108	46	0.1500	50.30	35.20	1.43
4	5	3.0	191	130	61	0.1560	60.00	40.80	1.47
5	6	2.9	225	155	70	0.1630	67.60	46.60	1.45
6	7	2.9	235	155	80	0.1540	74.75	49.15	1.52
7	10	2.8	248	166	82	0.1600	76.00	51.00	1.49
8	12	2.6	288	170	118	0.2025	69.60	41.20	1.69

Table 18

Zea Mays: 3 seedlings in each culture containing 350 cc. of ammonium nitrate solution whose concentration was 0.00246 N. Initial pH=5.4. Experimental duration: June 13–26, 1927. Temp. 10–25°C.

No.	days	pH	absorption (0.0001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00001 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	4.0	42	24	18	0.1485	13.87	7.93	1.75
2	2	3.6	78	59	19	0.1515	25.20	19.10	1.32
3	3	3.3	116	87	29	0.1510	37.60	28.20	1.33
4	5	3.0	167	122	45	0.1530	53.50	39.20	1.37
5	7	2.9	187	126	61	0.1545	59.30	40.10	1.48
6	8	2.8	208	122	86	0.1550	65.80	38.60	1.70
7	12	2.9	224	147	77	0.1690	64.98	42.70	1.52

Table 19

Zea Mays: 3 seedlings in each culture containing 350 cc. of ammonium nitrate solution whose concentration was 0.00063 N. Initial pH=5.4. Experimental duration: June 13–20, 1927. Temp. 10–25°C.

No.	days	pH	absorption (0.00001 N)		$[NH_4^{\cdot}]-[NO_3']$ (0.00081 N)	dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)		$\frac{NH_3}{NO_3}$
			NH_3	NO_3			NH_3-N	NO_3-N	
1	1	3.9	37	31	6	0.1480	12.25	10.40	1.19
2	2	4.0	47	43	4	0.1485	15.50	14.20	1.09
3	3	4.2	63	59	4	0.1460	21.13	19.75	1.07
4	5	4.6	63	61	2	0.1465	21.00	20.40	1.03
5	7	4.8	63	63	0	0.1460	21.13	21.13	1.00

Table 20

Zea Mays: 3 seedlings in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in each solution being equal. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 15 hours; April 4–5, 1928. Temp. 15–25°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	2.9	18	10	0.1430	6.22	3.42
2	4.2	4.1	26	17	0.1818	7.00	4.58
3	4.5	4.4	28	22	0.2100	6.53	5.13
4	5.0	5.0	20	1	0.1950	5.02	0.25
5	5.5	5.5	24	20	0.1935	6.06	5.06
6	6.0	6.0	28	22	0.1935	7.07	5.57
7	6.5	6.4	20	7	0.2165	4.52	1.58
8	7.0	6.8	28	14	0.1905	7.20	3.60
9	7.5	7.4	28	21	0.1825	7.50	5.65
10	8.0	7.8	22	18	0.1810	5.95	4.87
11	8.5	8.3	26	3	0.1685	7.55	0.87

Table 21

Zea Mays: 3 seedlings in each culture contatining 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in each solution being equal. Concentration of NH_4NO_3: 0.00354 N. Experimental duration: 24 hours; April 24–25, 1928. Temp. 20–30°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	24	21	0.1585	7.42	6.50
2	4.0	3.7	40	22	0.1725	11.38	6.25
3	4.6	4.2	46	35	0.1513	14.89	11.33
4	5.0	4.5	50	33	0.1785	13.70	9.05
5	5.5	5.4	46	33	0.1290	17.50	12.52
6	6.0	5.9	44	27	0.1660	12.99	7.97
7	6.5	6.3	34	7	0.1716	9.72	2.00
8	7.0	6.8	46	26	0.1740	12.94	7.32
9	7.5	7.2	38	21	0.1435	13.00	7.17
10	8.0	7.6	38	14	0.1670	11.14	4.11

Table 22

Zea Mays: 3 roots in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in each solution being equal. Concentration of NH_4NO_3: 0.00354 N. Experimental duration: 24 hours; April 24–25, 1928. Temp. 20–30°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	24	14	0.1410	8.35	4.87
2	4.0	3.8	24	14	0.1260	9.35	5.45
3	4.6	4.2	34	27	0.1463	11.35	9.00
4	5.0	4.7	28	7	0.1260	10.90	2.71
5	5.5	5.5	34	19	0.1238	13.48	7.53
6	6.0	5.9	36	29	0.1346	13.10	10.55
7	6.5	6.3	30	14	0.1183	12.40	5.80
8	7.0	6.9	38	27	0.1245	14.93	10.60
9	7.5	7.2	38	29	0.1267	14.70	11.20
10	8.0	7.8	24	7	0.1010	11.65	3.40
11	8.5	7.9	34	22	0.1175	14.18	9.15

Table 23

Zea Mays: 5 roots in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in each solution being equal. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 6 hours; May 5, 1928. Temp. 15°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	4	3	0.1174	1.67	1.25
2	4.0	3.9	8	5	0.1164	3.33	2.10
3	4.5	4.4	8	6	0.1526	2.53	1.93
4	5.0	4.9	6	4	0.1348	2.14	1.45
5	5.5	5.4	8	7	0.1300	3.01	2.62
6	6.0	6.0	12	12	0.1025	5.73	5.73
7	6.5	6.4	14	6	0.1180	5.81	2.48
8	7.0	7.0	24	15	0.1440	8.17	5.10
9	7.4	7.4	16	10	0.1300	6.02	3.77
10	8.0	8.0	16	7	0.0975	8.05	3.52
11	8.5	8.5	24	13	0.1237	9.50	5.15

Table 24

Zea Mays: 3 roots in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00356 N. Experimental duration: 24 hours; May 23–24, 1928. Temp. 15°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	2.9	20	14	0.0990	9.90	6.93
2	4.0	3.8	22	15	0.0906	11.90	7.70
3	4.5	4.4	24	17	0.1020	11.50	8.16
4	5.0	5.0	14	14	0.1000	6.85	6.85
5	5.5	5.5	16	14	0.0679	11.50	10.10
6	6.0	6.0	22	21	0.0920	11.70	11.20
7	6.4	6.3	26	15	0.1115	11.40	6.60
8	7.0	6.8	22	12	0.0935	11.51	6.30
9	7.4	7.3	22	15	0.0895	12.05	8.22
10	8.0	7.9	20	11	0.0920	10.65	5.27
11	8.5	8.0	28	17	0.1015	13.50	8.20

Table 25

Zea Mays: 3 roots in each culture contarning 350. cc. of solution (Naphosphate+NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00001 N. Experimental duration: 48 hours; May 23-25, 1928. Temp. 15°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	2.9	14	12	0.1010	6.79	5.82
2	4.0	4.0	16	15	0.1075	7.30	6.85
3	4.5	4.4	20	18	0.1000	9.80	8.82
4	5.0	5.0	10	10	0.0905	5.41	5.41
5	5.5	5.5	14	14	0.0872	7.87	7.87
6	6.0	5.9	20	20	0.0802	12.20	12.20
7	6.4	6.3	16	15	0.0831	9.45	8.85
8	7.0	6.9	22	19	0.0974	11.05	9.55
9	7.4	7.2	18	16	0.0900	9.80	8.70
10	8.0	7.8	20	11	0.0782	12.50	6.90
11	8.5	8.0	22	16	0.0870	12.40	9.00

Table 26

Zea Mays: 3 roots in each culture containing 350 cc. of solution (Na-phosphate+NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 12 hours; June 12–13, 1928. Temp. 20°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	12	13	0.0785	7.50	8.12
2	3.4	3.4	14	13	0.0870	7.88	7.32
3	3.8	3.7	10	13	0.0610	8.04	10.42
4	4.0	3.9	14	13	0.0696	9.85	9.15
5	4.2	4.2	10	13	0.0670	7.31	9.50
6	4.4	4.4	8	8	0.0705	5.56	5.56
7	4.6	4.5	8	8	0.0790	4.96	4.96
8	4.8	4.6	8	6	0.0790	4.96	3.75
9	5.0	4.9	10	6	0.0767	6.38	3.82
10	5.2	5.1	4	6	0.0727	2.70	4.05
11	5.4	5.4	10	8	0.0738	6.65	5.32
12	5.6	5.6	10	9	0.0740	6.64	5.95
13	5.8	5.8	10	10	0.0705	6.95	6.95
14	6.0	6.0	14	11	0.0825	8.32	6.54
15	6.2	6.2	12	7	0.0740	7.95	4.64
16	6.4	6.4	8	4	0.0760	5.15	2.58
17	6.6	6.6	12	6	0.0800	7.35	3.67
18	6.8	6.8	14	7	0.0970	7.08	3.54
19	7.0	6.9	12	7	0.0640	9.35	5.45
20	7.2	7.1	10	3	0.0725	6.75	2.03
21	7.4	7.4	12	5	0.0738	8.00	3.32
22	7.6	7.6	14	7	0.0665	10.30	5.15
23	7.8	7.7	18	5	0.0930	9.50	2.63
24	8.0	7.9	12	5	0.0663	8.90	3.70
25	8.2	8.1	16	8	0.0950	8.25	4.13
26	8.6	8.4	16	7	0.0760	10.30	4.51

Table 27

Zea May: 4 roots in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.0360 N. Experimental duaation: 12 hours; June 26–27, 1928. Temp. 15–18°C.

No.	pH		absorption (0.00001 N)		dry weignt of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	8	6	0.0655	5.95	5.20
2	3.2	3.2	10	7	0.0825	6.00	4.15
3	3.4	3.4	8	8	0.0785	4.90	4.90
4	3.6	3.6	8	8	0.0665	5.90	5.90
5	3.8	3.8	8	8	0.0790	4.96	4.96
6	4.2	4.2	9	7	0.0865	5.10	3.97
7	4.4	4.4	11	11	0.0885	6.09	6.09
8	4.6	4.6	10	10	0.0805	6.09	6.09
9	4.8	4.6	14	12	0.0980	7.00	6.00
10	5.0	4.9	10	10	0.0700	7.00	7.00
11	5.2	5.1	6	4	0.0980	3.00	2.00
12	5.4	5.4	10	10	0.0670	7.00	7.00
13	5.6	5.6	13	13	0.0912	7.00	7.00
14	5.8	5.8	10	7	0.0560	9.00	6.12
15	6.0	6.0	10	8	0.0802	6.12	4.90
16	6.2	6.2	10	8	0.0980	5.00	4.00
17	6.4	6.3	8	4	0.0980	4.00	2.00
18	6.6	6.6	12	8	0.0802	7.32	4.90
19	6.8	6.8	14	9	0.0750	9.15	5.87
20	7.0	7.0	16	6	0.0960	8.15	3.06
21	7.2	7.2	10	8	0.0970	5.00	4.02
22	7.4	7.4	11	7	0.0655	8.22	5.98
23	7.6	7.5	14	7	0.0850	8.10	4.03
24	7.8	7.6	14	5	0.0840	8.16	2.92
25	8.0	7.8	16	6	0.0960	8.16	3.06
26	8.2	8.0	12	7	0.0840	7.00	4.09
27	8.4	8.2	18	7	0.0675	13.08	5.10

Table 28

Zea Mays: 5 seedlings in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 20 hours; July 19–20, 1928. Temp. 15–30°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	23	7	0.1615	7.00	2.12
2	3.2	3.2	22	9	0.1340	8.05	7.00
3	3.4	3.4	26	23	0.1365	9.34	8.25
4	3.6	3.6	26	23	0.1275	10.03	8.88
5	3.8	3.8	26	23	0.1270	10.03	8.88
6	4.0	3.9	28	28	0.1350	10.15	10.15
7	4.2	4.2	28	23	0.1240	11.07	9.10
8	4.4	4.2	28	28	0.1245	11.02	11.02
9	4.6	4.1	31	23	0.1400	10.85	8.05
10	4.8	4.4	30	22	0.1315	11.18	8.20
11	5.0	4.6	32	23	0.1110	14.10	10.15
12	5.2	4.8	32	15	0.1215	12.90	6.05
13	5.4	5.2	28	13	0.1245	11.00	5.12
14	5.6	5.6	33	23	0.1245	13.00	9.05
15	5.8	5.8	38	20	0.1245	14.96	7.90
16	6.0	5.9	38	22	0.1100	16.90	9.80
17	6.2	6.1	45	23	0.1390	15.85	8.10
18	6.4	6.3	30	17	0.1210	12.15	6.89
19	6.6	6.6	36	24	0.1180	14.93	9.95
20	6.8	6.7	43	22	0.1300	16.20	8.30
21	7.0	6.9	41	21	0.1250	16.00	8.25
22	7.2	7.1	39	17	0.1050	18.20	7.94
23	7.4	7.4	42	16	0.1290	15.97	6.08
24	7.6	7.6	42	23	0.1280	16.08	8.80
25	7.8	7.5	33	18	0.1150	14.05	8.10
26	8.0	7.6	39	17	0.1190	16.05	7.00
27	8.2	7.7	39	18	0.1275	14.98	6.92
28	8.4	7.8	35	10	0.1150	14.90	4.25
29	8.6	7.9	44	10	0.1260	17.10	3.90

Table 29

Zea Mays: 4 seedlings in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3 : 0.00360 N. Experimental duration: 24 hours; July 31–Aug. 1, 1928. Temp. 20–30°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	19	19	0.1165	8.00	8.00
2	3.2	3.2	19	19	0.1165	8.00	8.00
3	3.4	3.4	19	19	0.1165	8.00	8.00
4	3.6	3.6	19	19	0.1210	7.55	7.55
5	3.8	3.8	23	21	0.1130	10.00	9.10
6	4.0	4.0	23	21	0.1130	10.00	9.10
7	4.2	4.0	24	21	0.1300	9.05	7.92
8	4.4	4.1	24	19	0.1160	10.13	8.03
6	4.8	4.4	37	24	0.1275	14.20	9.22
10	5.0	4.6	34	24	0.1285	12.98	9.15
11	5.2	5.0	27	17	0.1210	10.92	6.88
12	5.4	5.2	23	19	0.1035	10.90	9.00
13	5.6	5.5	26	24	0.0970	13.13	12.10
14	5.8	5.8	33	21	0.1340	12.05	8.05
15	6.0	6.0	36	20	0.1255	14.06	7.81
16	6.2	6.2	32	20	0.1215	12.90	7.90
17	6.4	6.4	28	18	0.1245	11.02	7.10
18	6.6	6.6	32	25	0.1185	13.22	10.32
19	6.8	6.7	38	20	0.1240	15.00	7.90
20	7.0	6.8	44	20	0.1105	18.15	8.87
21	7.2	7.0	40	20	0.1230	15.95	7.96
22	7.4	7.3	43	18	0.1000	21.08	8.82
23	7.6	7.5	44	18	0.1270	16.95	6.94
24	7.8	7.6	46	15	0.1250	18.00	5.88
25	8.0	7.7	36	13	0.1260	14.00	5.10
26	8.2	7.8	42	11	0.1370	15.00	3.94
27	8.4	7.8	40	11	0.1285	15.15	4.20
28	8.6	8.0	33	10	0.1160	13.90	3.80

Table 30

Zea Mays: 4 seedlings in each culture containing 350 cc. of solution (Na-phosphate + NH_4NO_3), the amount of cations in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 22 hours; Sept. 1–2, 1928. Temp. 15–20°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initiat	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.2	3.3	18	38	0.1240	7.11	15.00
2	3.4	3.5	18	38	0.1240	7.11	15.00
3	3.6	3.7	18	42	0.1450	6.08	14.20
4	4.0	4.0	29	29	0.1305	10.88	10.88
5	4.2	4.2	27	25	0.1110	11.92	11.02
6	4.4	4.2	25	25	0.1190	10.30	10.30
7	4.6	4.3	24	21	0.1280	9.18	8.04
8	5.0	4.8	25	18	0.1225	10.00	7.20
9	5.2	5.0	19	19	0.1155	8.07	8.07
10	5.4	5.1	14	12	0.1180	5.82	4.97
11	5.6	5.4	30	15	0.1490	9.86	4.94
12	5.9	5.9	29	14	0.1175	12.06	5.83
13	6.2	6.2	32	15	0.1515	10.03	4.85
14	6.3	6.3	27	5	0.1175	11.25	2.08
15	6.5	6.4	48	14	0.1675	14.06	4.10
16	6.7	6.6	37	14	0.1290	14.05	4.95
17	6.8	6.8	37	12	0.1415	12.80	4.15
18	7.0	6.9	38	12	0.1170	15.95	5.02
19	7.2	7.0	38	12	0.1175	15.90	5.00
20	7.4	7.1	57	9	0.1460	19.10	3.02
21	7.6	7.3	55	15	0.1490	18.05	4.93
22	7.8	7.4	50	12	0.1450	16.90	4.05
23	8.0	7.5	56	12	0.1440	19.00	4.08
24	8.2	7.6	38	10	0.1200	15.95	4.08
25	8.3	7.6	45	10	0.1225	18.00	4.00
26	8.4	7.7	45	5	0.1230	17.90	1.99

Table 31

Zea Mays: 5 seedlings in each culture containing 350 cc. of solution (Na-phosphate+NH_4NO_3), the amount of cations in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 24 hours; Sept. 29-30. 1928. Temp. 10-15°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.0	9	13	0.1105	3.99	5.76
2	3.2	3.2	13	21	0.1275	5.00	8.07
3	3.4	3.4	8	16	0.1000	3.92	7.84
4	3.6	3.6	15	16	0.0930	7.90	8.90
5	3.8	3.8	13	20	0.0900	7.07	10.09
6	4.0	4.0	18	20	0.1080	8.16	9.09
7	4.2	4.2	17	20	0.1200	6.95	8.15
8	4.4	4.4	14	20	0.0990	6.93	9.90
9	4.6	4.5	15	20	0.1245	5.90	7.87
10	4.8	4.8	16	18	0.1280	6.12	6.88
11	5.0	5.0	11	13	0.1070	5.04	5.95
12	5.2	5.2	20	20	0.1205	8.13	8.13
13	5.4	5.3	26	20	0.1280	9.95	8.05
14	5.8	5.8	25	25	0.1200	10.20	10.20
15	6.0	6.0	19	19	0.1310	7.10	7.10
16	6.2	6.2	16	16	0.1320	5.94	5.94
17	6.4	6.3	16	14	0.1140	6.88	6.02
18	6.6	6.5	20	16	0.1110	8.83	7.06
19	6.8	6.7	23	13	0.1255	8.98	5.07
20	7.0	7.0	24	12	0.1185	9.95	4.97
21	7.2	7.2	20	11	0.1090	9.00	4.95
22	7.4	7.3	25	8	0.1330	9.20	2.95
23	7.6	7.4	24	7	0.0910	12.95	3.76
24	7.8	7.5	26	8	0.1270	9.98	3.03
25	8.0	7.7	25	7	0.1120	10.94	3.06
26	8.1	7.7	33	6	0.1460	11.06	2.10
27	8.2	7.8	23	7	0.1160	9.72	2.96
28	8.4	7.9	30	3	0.1215	12.10	1.21

Table 32

Zea Mays: 6 seedlings in each culture containing 350 cc. of solution (oxalate mixture + NH_4NO_3), the amount of cations in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00358 N. Experimental duration: 24 hours; Oct. 30–31, 1928. Temp. 10–20°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.5	3.6	10	16	0.1550	3.16	5.05
2	4.0	4.0	12	18	0.1480	3.97	5.95
3	4.2	4.3	18	18	0.1815	4.86	4.86
4	4.5	4.4	20	17	0.1630	6.01	5.10
5	4.8	4.8	20	20	0.1620	6.05	6.05
6	5.0	5.1	28	24	0.1700	8.07	6.92
7	5.2	5.2	16	12	0.1500	5.22	3.92
8	5.4	5.3	24	13	0.1665	7.06	4.12
9	5.6	5.4	26	23	0.1600	7.96	7.05
10	5.8	5.7	27	18	0.1660	7.97	5.02
11	6.0	5.4	33	21	0.1600	10.10	6.12
12	6.2	5.7	32	21	0.1600	9.80	6.12
13	6,4	5.8	32	18	0.1720	9.10	5.13
14	6.6	6.0	29	18	0.1775	8,00	4.95
15	7.2	5.8	28	11	0.1740	7.90	3.09
16	7.8	6.1	28	7	0.1740	7.00	1.95
17	8.4	6.4	28	5	0.1370	0.00	1.79
18	9.0	7.6	30	1	0.1485	9.90	0.33

Table 33

Zea Mays: 4 seedlings in each culture containing 350 cc. of solution (oxalate mixture + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: 24 hours; Dec. 24–25, 1928. Temp. 15–25°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initiat	final	NH_3	NO_3		NH_3-N	NO_3-N
1	4.2	4.1	5	9	0.0640	3.83	7.00
2	4.4	4.4	5	9	0.0640	3.83	7.00
3	4.6	4.6	7	10	0.0790	4.90	7.00
4	4.8	4.8	10	11	0.0555	8.83	9.70
5	5.0	5.0	14	11	0.0750	9.15	7.20
6	5.2	5.2	9	11	0.0765	5.77	7.05
7	5.4	5.6	16	13	0.0715	10.96	8.92
8	5.6	6.0	18	13	0.0625	14.10	10.20
9	6.0	6.2	21	14	0.0730	14.10	9.40
10	6.4	6.4	15	13	0.0675	10.88	9.45
11	6.6	6.4	16	16	0.0650	12.05	12.05
12	6.8	6.8	17	14	0.0550	15.15	12.45
13	7.0	6.8	17	11	0.0635	13.10	7.75
14	7.3	7.0	20	12	0.0815	12.02	7.20
15	7.6	6.8	20	7	0.0550	17.85	6.25
16	8.0	6.8	22	4	0.0600	17.98	3.27
17	8.5	7.0	22	5	0.0710	15.15	2.82

Table 34

Zea Mays: 6 seedlings in each culture containing 350 cc. of solution (KNOP + Na-phosphate), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00620 N. Experimental duration: 48 hours; June 18–20, 1929. Temp. 10–20°C.

No.	pH		absorption (0.0001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3	NO_3
1	3.0	3.2	41	47	0.1425	13.75	16.18
2	3.4	3.8	64	73	0.1565	20.00	27.50
3	3.8	3.9	70	73	0.1620	21.15	22.00
4	4.3	4.2	80	73	0.1470	26.65	24.20
5	4.8	4.4	110	87	0.1370	39.35	31.00
6	5.0	4.6	120	100	0.1540	38.20	31.80
7	5.2	5.0	110	100	0.1420	37.90	34.50
8	5.4	5.2	114	100	0.1610	34.70	30.40
9	5.6	5.4	118	100	0.1665	34.75	29.40
10	5.8	5.6	116	113	0.1605	35.40	34.40
11	6.0	5.8	130	113	0.1765	36.20	31.40
12	6.2	6.0	126	126	0.1495	41.30	41.30
13	6.4	6.3	106	87	0.2040	25.45	20.90
14	6.6	6.4	148	100	0.1700	42.60	28.90
15	6.8	6.7	120	100	0.1485	39.60	33.00
16	7.0	6.7	136	87	0.1870	35.65	22.85
17	7.2	6.9	150	87	0.1585	46.40	26.80
18	7.5	7.0	126	73	0.1550	39.80	23.00
19	7.8	7.1	168	87	0.2045	40.35	20.90

Table 35

Zea Mays: 6 seedlings in each culture containing 350 cc. of solution (KNOP+Na-phosphate), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00620 N. Experimental duration: 72 hours; June 18–21, 1929. Temp. 10–20°C.

No.	pH		absorption (0.00001 N)		dry weight of the root (gm.)	N-absorbed per 1 gm. of dry root (mg.)	
	initial	final	NH_3	NO_3		NH_3-N	NO_3-N
1	3.0	3.2	102	113	0.1430	34.90	38.80
2	3.4	3.6	130	140	0.1640	38.90	41.80
3	3.8	3.8	157	153	0.1725	44.60	43.25
4	4.3	4.0	125	100	0.1635	37.45	30.00
5	4.8	4.0	137	127	0.1930	34.80	31.40
6	5.0	4.6	137	127	0.2060	32.60	30.20
7	5.2	4.8	147	127	0.2080	34.60	29.80
8	5.4	5.0	137	113	0.2015	33.20	27.45
9	5.6	5.2	164	127	0.1735	46.30	35.90
10	5.8	5.4	144	127	0.1680	42.00	37.10
11	6.0	5.7	185	153	0.1720	52.70	43.50
12	6.2	6.0	154	140	0.1830	41.20	37.50
13	6.4	6.2	185	140	0.1950	46.50	35.20
14	6.6	6.5	170	113	0.1810	46.00	30.70
15	6.8	6.6	184	113	0.1895	47.75	25.25
16	7.0	6.8	202	140	0.1720	57.50	39.90
17	7.2	7.0	180	113	0.2000	44.10	27.70
18	7.5	7.0	234	153	0.2195	52.00	34.00
19	7.8	7.1	175	87	0.1655	52.00	24.20

Table 36a

Zea Mays: 6 seedlings in each culture containing 350. cc. of solution (Na-phosphate + NH_4NO_3), the amount of anions in these solutions being equal to each other. Concentration of NH_4NO_3: 0.00360 N. Experimental duration: Nov. 9–23, 1928. Temp. 15–25°C.

No.	pH							dry weight of plants (gm.)	
	initial	daily changes							
		3	6	8	10	12	14	shoot	root
1	3.0	3.0	2.9	2.9	3.0	3.0	3.0	0.1800	0.0460
2	3.6	3.6	3.5	3.5	3.5	3.5	3.5	0.1945	0.0915
3	4.0	3.9	3.8	3.8	3.8	4.0	4.0	0.2290	0.0760
4	4.2	4.2	4.0	4.1	4.2	4.4	4.2	0.2360	0.0830
5	4.4	4.4	4.1	4.2	4.3	4.5	4.4	0.2020	0.0770
6	4.6	4.4	4.2	4.4	4.6	4.8	4.6	0.2370	0.0810
7	5.0	4.6	4.4	4.8	4.9	5.0	5.0	0.2400	0.0900
8	5.2	4.8	4.8	5.0	5.1	5.2	5.2	0.2420	0.1020
9	5.4	5.2	5.2	5.2	5.2	5.4	5.4	0.1940	0.0650
10	5.6	5.4	5.4	5.5	5.5	5.6	5.6	0.2240	0.0855
11	5.8	5.7	5.8	5.8	5.8	5.8	5.8	0.2605	0.0940
12	6.0	6.0	6.0	6.0	6.0	6.0	6.0	0.1995	0.0900
13	6.2	6.1	6.2	6.2	6.2	6.2	6.2	0.1655	0.0775
14	6.4	6.3	6.3	6.3	6.3	6.4	6.4	0.1990	0.0860
15	6.6	6.6	6.5	6.0	6.0	6.2	6.2	0.2055	0.1015
16	6.8	6.8	6.8	6.8	6.8	6.8	6.8	0.1470	0.0630
17	7.0	7.0	7.0	7.0	7.0	7.0	7.0	0.1340	0.0515
18	7.2	7.2	7.2	7.2	7.2	7.2	7.2	0.1295	0.0425
19	7.6	7.4	7.4	7.5	7.5	7.5	7.5	0.1175	0.0355
20	8.0	7.6	7.7	7.8	7.8	7.8	7.8	0.0955	0.0460
21	8.6	8.0	8.2	8.4	8.5	8.4	8.4	1.0820	0.0260

Table 36b

No.	initial pH	ammonia absorbed (0.00001 N) during the interval (days)							N-absorbed per 1 gm. of dry root (mg.)
		3	6	8	10	12	14	total	
1	3.0	18	40	12	10	0	0	80	85.25
2	3.6	30	40	15	15	5	0	105	72.10
3	4.0	35	40	20	18	8	0	121	78.00
4	4.2	35	43	20	22	6	0	126	74.50
5	4.4	35	38	15	20	6	0	114	72.60
6	4.6	35	42	17	20	2	0	116	70.40
7	5.0	43	48	20	20	8	5	144	78.50
8	5.2	42	43	20	20	8	2	135	64.80
9	5.4	35	37	22	22	2	2	120	90.50
10	5.6	44	45	22	25	5	4	145	83.20
11	5.8	54	59	25	27	10	−2	173	90.00
12	6.0	44	43	18	28	18	4	155	84.45
13	6.2	42	40	13	18	8	0	121	76.50
14	6.4	37	35	16	22	15	2	127	72.40
15	6.6	45	40	24	26	18	0	153	73.90
16	6.8	43	30	16	22	0	-5	111	86.50
17	7.0	38	32	15	8	8	0	101	96.00
18	7.2	38	33	13	8	2	0	94	100.85
19	7.6	52	33	10	14	8	2	119	164.50
20	8.0	52	13	6	5	8	0	84	89.50
21	8.6	42	13	10	10	10	10	95	179.00

Table 37a

Zea Mays: 6 seedlings in each culture containing 350 cc. of nutrient solution (complete solution + Na-phosphate), the amount of anions in these solutions being equal to each other. The nutrient solution was renewed every 24 hours. Concentration of NH_4NO_3: 0.00650 N. Experimental duration: Aug. 20–31, 1929. Temp. 15–25°C.

No.	PH												dry weight of plants (gm.)	
	initial	daily changes												
		1	2	3	4	5	6	7	8	9	10	11	shoot	root
1	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.3470	0.0655
2	3.5	3.5	3.5	3.5	3.4	3.5	3.5	3.4	3.5	3.5	3.5	3.4	0.3990	0.1000
3	3.8	3.7	3.7	3.8	3.7	3.6	3.6	3.7	3.7	3.7	3.7	3.7	0.3995	0.1060
4	4.5	4.5	4.5	4.5	4.4	4.2	4.2	4.2	4.2	3.8	4.2	4.2	0.5465	0.1325
5	5.0	4.9	4.8	4.9	4.8	4.7	4.8	4.7	4.7	4.7	4.8	5.0	0.5010	0.1230
6	5.2	5.2	5.2	5.2	5.0	5.0	5.1	4.9	4.9	5.0	5.0	5.1	0.4970	0.1250
7	5.4	5.4	5.3	5.4	5.2	5.2	5.2	5.2	5.1	5.1	5.2	5.2	0.4710	0.1080
8	5.6	5.5	5.5	5.5	5.4	5.4	5.4	5.4	5.5	5.4	5.5	5.4	0.5875	0.1230
9	5.8	5.7	5.7	5.6	5.6	5.5	5.6	5.6	5.6	5.7	5.7	5.7	0.5855	0.1250
10	6.0	5.9	5.9	5.9	5.8	5.9	5.9	5.9	5.9	5.9	6.0	5.9	0.5290	0.1255
11	6.2	6.1	6.1	6.1	6.0	6.0	6.1	6.1	6.1	6.1	6.2	6.1	0.4745	0.1290
12	6.4	6.4	6.4	6.4	6.2	6.4	6.4	6.3	6.3	6.4	6.4	6.4	0.5465	0.1355
13	6.6	6.6	6.6	6.6	6.5	6.4	6.6	6.5	6.6	6.6	6.6	6.6	0.5700	0.1380
14	6.8	6.8	6.7	6.7	6.6	6.8	6.8	6.8	6.8	6.8	6.8	6.8	0.5545	0.1320
15	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	0.5595	0.1300
16	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	0.4930	0.1100
17	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	0.5695	0.1100
18	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	0.4045	0.0785

Table 37b

No.	initial pH	ammonia absorbed (0.00001 N) during the interval (days)												N-absorbed per 1 gm. of dry root (mg.)
		1	2	3	4	5	6	7	8	9	10	11	total	
1	3.0	9	8	12	13	17	12	12	10	10	10	10	123	99.50
2	3.5	11	15	14	15	22	20	20	15	20	15	14	181	88.70
3	3.8	12	15	20	17	25	20	20	15	25	20	20	209	96.50
4	4.5	13	18	26	35	32	30	25	20	33	22	25	279	103.00
5	5.0	20	25	30	33	32	30	24	25	25	20	20	284	113.20
6	5.2	15	23	25	45	40	35	40	30	25	20	20	318	124.80
7	5.4	20	28	32	33	35	30	25	20	20	10	15	268	121.50
8	5.6	20	27	33	33	38	30	27	25	23	14	17	287	114.20
9	5.8	23	28	32	35	42	35	30	30	25	18	23	321	125.50
10	6.0	22	27	35	40	48	35	42	30	25	20	23	345	134.80
11	6.2	28	30	37	52	38	30	40	25	25	25	20	350	133.00
12	6.4	25	25	37	55	55	44	40	35	30	25	36	397	143.50
13	6.6	30	34	42	68	67	54	50	55	35	30	30	495	176.00
14	6.8	30	35	42	58	55	50	47	45	30	30	35	457	169.50
15	7.0	35	37	50	55	55	50	40	40	30	30	25	447	168.50
16	7.4	30	40	44	45	48	35	40	35	35	30	25	407	181.00
17	7.6	35	40	43	42	45	35	40	35	35	30	30	410	182.60
18	8.0	20	35	30	32	35	15	33	35	35	30	30	330	202.80

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Plate I.

Experiment 38: Growth of seedlings of *Zea Mays* in the culture solutions of varying pH-values.

Loo Phot.

Errata

Loo T-L.: Studies on the absorption of ammonia and nitrate by the root of *Zea Mays*-seedlings, in relation to the concentration and the actual acidity of culture solution. Journ. Fac. Agr. Hokkaido Imp. Univ. Vol. XXX, Pt. 1. 1931.

page	line	incorrect	correct
2	6	date	data
3	19	Kjehedahl	Kjeldahl
"	20	"	"
"	27	"	"
5	16	LUB	LUBS
6	16	origodynamically	oligodynamically
7	3	differings	differing
8	35	experiment	experiments
10	36	experiment	experimental
11	8	wich	which
"	19	Tableg	Table
"	34	solution	solutions
15	11	$[NH_4]$-$[NO_3]$	$[NH_4^{\cdot}]$-$[NO_3']$
"	26	cations	cation
19	32	othe*r*	other
20	9	later	latter
"	20	soltion	solution
25	19	culturs	culture
27	23	result	results
"	25	date	data
28	32	excrction	excretion
31	37	solutionions	solution
32	14	shows	show
33	10	sbsorption	absorption
35	24	protplasm	protoplasm
"	25	sutudies	studies
"	31	ph	pH
36	7	experriment	experiment
38	1	phosphate, mixture,	phosphate mixture,
40	2	date	data
"	14–15	Text-fiig.	Text-fig.
40	23	§en	seen
42	8	a	are
"	21	recorgnized	recognized
44	2	laboratoly	laboratory
"	13	amonia	ammonia
45	6	eqnal	equal
46	28	sistem	system
47	22	perparation	preparation
50	12	Text-fiig.	Text-fig.
"	32	againts	against
58	3	Text-fiig.	Text-fig.
59	Table, column 6	NaH_2PO_4	Na_2HPO_4
"	" " 8	100	1000
61	8	Text-fiig	Text-fig
63	11	deplopment	development
"	13	ouly	only
"	32	tne	the
64	5	valuse	values
"	16	ta	to
66	27	Text-fiig.	Text-fig.
71	2	cvrves	curves
76	9	anology	analogy
"	22	protien	protein
80	14	phonomenon	phenomenon
82	13	absobed	absorbed
83	31	recearches	researches
84	1	**VI. Experimental data**	**Experimental data**
"	Table 1	$[NH'_4]$-$[MO'_3]$	$[NH_4^{\cdot}]$-$[NO_3']$
99	2	Naphosphate	Na-phosphate
101	5	duaation	duration