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A study of absorption and excretion of potassium and calcium by the roots of barley in different solution media and changes in hydrogen-ion concentration.

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A Study of Absorption and Excretion of Potassium
And Calcium by the Roots of Barley in Different
Solution Media & Changes in Hydrogenion Concentration

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A STUDY OF ABSORPTION AND EXCRETION OF POTASSIUM AND CALCIUM BY THE ROOTS OF BARLEY IN DIFFERENT SOLUTION MEDIA AND CHANGES IN HYDROGEN-ION CONCENTRATION

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I INTRODUCTION

Since it is known that plants take up salts, and that these salts are absolutely necessary for their life, it became important to learn the mechanism of this salt absorption. The original question, the cause of many investigations, was the following: How do roots take up the salts from the surrounding solution, what principles are involved, and what factors do affect this phenomenon?

The many experiments conducted concerning this problem of salt absorption show surprisingly uniform results. They all prove that the plants take up nutrient elements in the ionic form; cations and anions are taken up separately. This theory of a separate absorption of ions results in a difficulty of considerable importance; the fact that each ion, unlike the molecules, has an electrical charge of certain amount and kind. An absorption at random of single ions is thus out of question, since tremendous potentialities, under such circumstances, consequently must occur. Such a stress is physiologically and chemically impossible. It is clear, therefore, that the "selective properties" of a plant for ions is entirely due to a constantly changing equilibrium between the physio-chemical condition of the cell and the forces acting on it.

The maintenance of such an electrostatic equilibrium during the ion uptake is possible in two ways:

either ion pairs of opposite charge are taken up, or in the case of one-sided absorption of positively and negatively charged ions, equivalent amounts of equally charged ions are given off. In both cases a balance of the electrostatic charges takes place. The uptake of ionic pairs equals practically (not theoretically) an absorption of molecules.

Since culture solutions and distilled water were exclusively used in this work, one has to consider the effects caused by the ions of the water. It is confirmed by many investigators (43, 44) that changes in pH go parallel with a one-sided increased uptake of ions. If more anions than cations are absorbed by the roots of the plant, the hydrogen ion concentration of the medium decreases, and vice versa.

It was mentioned before, (the second possibility for the maintenance of the electrostatic balance) that an equivalent excretion of equally charged ions may occur. One cannot, however, determine whether the change in pH is due to a migration of the OH-ion with the cation (uptake of ion pairs), or to an excretion of H-ions (ionic exchange). Both lead to the same result, a relative decrease of OH-ions, and consequently an increase of H-ions, respectively, in the medium. Fundamentally an ionic exchange is very well possible, and undoubtedly occurs to a certain degree.

This investigation is concerned with the absorption and excretion of calcium and potassium by barley roots in different solution media, and its effect in changes in hydrogen-ion concentration.

II   REVIEW OF LITERATURE

The question of absorption and excretion of ions by plant roots is not new, many volumes having been written on the subject. The reasons for these phenomena are, however, still unexplained. All investigations reported so far deal with single occurrences within the entire phenomenon of root absorption and cannot be considered collectively. They are accidental descriptions based on hypothetical conclusions. In none of the works published could a close correlation with the metabolism of the experimental plant be found. According to Achromeiko (1) the conclusions are based on qualitative rather than on quantitative proof.

Justus von Liebig (2) and J. Sachs (3), as the first investigators, approached the problem of excretion by the roots scientifically. They were mainly interested in the acid properties of such excretions. They thought of the possibility that such acid compounds might be able to render partly insoluble inorganic salts in the soil available for plant use.

Fr. Czapek (4) did not see, in his work "Wurzelaus-scheidungen", any chemical significance in such root excretions. Through qualitative analyses of a relatively large number of plants he found that roots excrete nitrogen,

potassium, phosphoric acid, sulphuric acid, calcium, and other elements. In his experiments Czapek used young roots and brought them in contact with distilled water, or hung the experimental plants into a room saturated with moisture. In the latter experiment droplets occurred at the roots in which dissolved mineral materials could be found. Czapek explained the appearance of these droplets as being due to the change of the turgor within the plant cell under the influence of the low pressure of the saturated atmosphere.

Wilson (5) assumed that the excretion of plant sap by the roots is due to a change of osmotic pressure within the nectaries under the influence of an accumulation of sugar.

The red coloration of litmus paper when dipped into the solution excreted by the roots was explained by Czapek (5) as being due to acid potassium phosphate given off by the roots. In the majority of cases, however, the droplets at the roots are neutral in reaction.

Macaire-Princep (6), as far as it is known, was the first investigator who used water cultures in his examinations of root excretions. He used vessels filled with a) distilled water, b) sodium chloride, c) limewater, d) lead acetate. Plants were grown alternating in each of the four solutions, finishing the cycle with the distilled water. After a short period he was able to find in the

distilled water small quantities of the elements he applied
to the culture solutions. His results, however, were not
accepted, since the reappearance of the elements was ascribed
completely to excretion by dead roots. Injuries to the roots
were caused by physiologically unbalanced salt solution of
sodium chloride and lead acetate.

E. Schulze and W. Umlaut (7) observed in their
experiments that lupine plants, after standing in distilled
water for thirteen days, excreted organic and inorganic
materials. In this work, again, the determination of whether
living or dead roots are the reasons for the appearance of
such substances in distilled water was neglected.

W. Knop (8) found that leguminosae excrete con-
siderable amounts of phosphoric acid.

According to R. Kon (9), excretions by the roots
do not occur at all. There are only apparent root excre-
tions caused by the electrolytic action of the roots upon
the surrounding salt solutions. The roots of every plant
act like electrodes, dissociating the surrounding salts
into positively and negatively charged ions and attracting
acids. The accumulation of acid near the roots gives the
impression that it is excreted by the roots.

I. Stoklasa and A. Ernest (10) investigated the
excretion of carbon dioxide and different other organic

acids by the roots of many different plants. Oxygen was
supplied in varying amounts. They found that free mineral
acids and potassium monophosphate never could be detected.

Th. Pfeiffer and E. Blank (11) started an experi-
ment based on the fact that leguminosae are able to use the
phosphoric acid of raw phosphate much better and more
readily than graminaceae. The positive results in sand
cultures were explained, on the basis that the roots of
leguminosae excrete, besides carbon dioxide, other acids
which then work on the raw phosphate and render it soluble.

Wheat was used by O. F. Tueva (12) and the
excretion of calcium studied. Plants of different ages,
grown in complete culture solutions, were put into distilled
water acidified by a buffer solution. The giving off of
calcium was much higher in the more acid medium, and very
often stopped completely at a pH of 6.5 - 7.5. The explana-
tion for the minimum excretion at a high pH is that the
protein of the wheat is in an isoelectric condition.

In all of the listed works by different investiga-
tors, the excretion of mineral elements by the roots was
considered to be due entirely to differences in osmotic
pressure of these elements within the roots or in the medium.
It is understood and clear that the roots of any living plant
are able to take up all elements that are present in a

nutrient solution, the osmotic pressure will be greatly decreased. The consequence is a tendency to bring the osmotic pressure of the roots and the new medium to an equilibrium. To accomplish this, elements have to be given off into the surrounding medium.

This exosmosis of nutrient elements under such conditions is a quite natural reaction of the plant roots. However, such an excretion of mineral elements is just as remote from the natural physiological functions of the plant as is the bleeding of an injured animal from the natural functions of the animal organisms. Apparently it is a fact that plants are subject to exosmosis no matter what their age. There is only a little fluctuation when instead of the whole plant one takes only the root system (12).

A nutrient solution of such concentration can be used that the roots of any plant placed into it are able to assimilate or to excrete elements present in the solution. That this is correct was shown by an experiment conducted by I. Puge (13). The growing of wheat in a solution, containing less than 1 mgr. of phosphoric acid in 1000 cc of water, resulted in an exosmosis of phosphoric acid from the roots. Concentrations of more than 1 mgr. showed an assimilation of phosphoric acid by the wheat. The same phenomenon was reported of calcium. There again, it was

entirely due to the concentration of the culture solution (14).

Not only healthy living plants, but single parts of plants, are able to absorb elements. Walter Stiles (14) showed that pieces of potato tuber and of turnip, when brought into connection with salt solutions of higher osmotic pressure than the plant sap, absorbed ions of varying amounts. Stiles pointed out that there is an equilibrium exchange, an exchange of different ions for the ones taken up from the salt solution, until an equilibrium between the plant sap and the salt solution is reached.

From this short review of the mentioned investigations one can clearly see that the question of "root excretion" is far from being solved. As a matter of fact, it is still in its first steps. There is no doubt that the plants lose mineral materials when subjected to the rinsing effects of water. But it is still uncertain whether this phenomenon is caused by the normal physiological activities of the plants (in other words, if there is an excretion of ions under growing conditions), or if it occurs only when the plant roots are brought into contact with water.

In one instance this question seems to be completely clarified. Exosmosis is a natural reaction of every plant cell, dead or alive, to bring the plant sap and the

medium to a physiological and chemical equilibrium. But what happens when medium and plant sap are in proper electrostatic balance when the experiment is started? Is there an absorption, only, of the necessary elements from the solution, with a consequent lowering of the concentration of the salt solution and a decrease is osmotic pressure? Physiologically, this would be completely impossible. Or is there an exchange of ions, an appearance of ions in the solution, given off by the roots in an exchange for the ions taken up for the metabolism? Is such an exchange of ions proportional: that is, an excretion of two potassium ions for every calcium ion absorbed?

Such questions inevitably arise, and are of utmost importance in solving the problem of root excretions.

Uptake and excretion of ions

"First, one has to consider that all salts are definitely taken up by the plant." This was the final answer by Jost (15) after long experiments concerning the absorption of a great number of nutrient salts by roots of higher plants.

After this introductional statement one has first to consider whether he is justified in speaking of an absorption of salts. Nathansohn (16) modified the statement by explaining that ions, and not molecules, are absorbed.

Analyses show that cations and anions are absorbed from
salt solutions as well as from nutrient solutions, but not
in equivalent amounts.

Keller (17) concluded that, since weak acids or
bases penetrate the cells faster than strong acids or bases,
molecules are taken up easier than ions. In this case it
remains to be determined if the undissociated acid and base
molecules are the decisive factors, or if absorption is due
to a particular behavior of the H-ions and OH-ions.

Osterhout (18) in 1929 developed a series of
formulae which make it possible, by analyses, to determine
arithmetically whether ions or molecules were absorbed.
Pantanelli (19), on the other hand, insists that the con-
trary is true. Salts are absorbe the faster the more they
are dissociated. Further, he states, if one holds the
assumption of an ionic absorption, ionic pairs must be taken
up, and that the H-ions or OH-ions bring about a shifting
of the charge within the cell and thus open a passage for
increased uptake of opposite charged ions.

Brooks (20) argued for an absorption of ions,
stating that the "selective ion absorption" is connected
with electrostatic forces.

The fact that cations and anions of a salt are,
as a rule, not absorbed in equivalent proportions is not a

definite proof of a separate intake of ions, but it is a suggestive indication. Investigations by Pantanelli (21), Stiles (22) and Redfern (23), resulted in proof of an increased uptake of anions over cations from salt solutions of high concentrations, and nearly an equivalent absorption of ions from low concentrations.

Hoagland (24) and Davis (25) proved that the absorption occurs mostly from the place of lower to the place of higher concentration, which was against the principle of "osmosis". In view of this result, the older assumption by Stiles (22b) of "osmosis" cannot be regarded any longer as being correct.

The question of excretion of ions, occurring in exchange for ions needed by the plants, was investigated by many workers. Qualitatively, an excretion of mineral elements, particularly of calcium and potassium, was observed by Redfern (23) and Stiles (22). This is, however, not a proof, that the observed excretion of ions is an exchange for an equivalent amount of equally charged ions from the solution. It proves, however, quite contrary to previous assumptions, that plants are able to give off elements once taken up. Johnson (26) states that this is caused entirely by injured and dead roots.

An ionic exchange is very well possible, at least

to a certain degree, and not only by healthy plants but by plants with injured cells. Quantitative determinations by Hoagland (24), of the absorption and excretion of salts by barley plants, gave surprising proportional results.

Maiwald (21) considered it most probable that "die Naehrstoffaufnahme durch die Pflanze dem Hauptgrundsatz des Ionenaustausches unterworfen ist". Such statements can be found quite frequently in recent published works (43, 44, 23).

All those experiments in which the uptake of salts was determined by measuring the conductivity of the solution argue against the assumption of a proportional inoic exchange, (better, against the maintenance of the original proportions by ionic exchange). Measurements of conductivity give a correct answer to the ionic concentration of a solution. If ions would be exchanged constantly, the conductivity of the solution should necessarily not be changed. Jenny (28), Bode (29), and Dahm (30) detected changes in conductivity of solutions under the influence of plants, and they explained their results by a one-sided absorption or excretion of ions and consequently a change in ionic concentration.

III EXPERIMENTAL METHODS

Culture solutions

Shive's 1/1 complete culture solution R5S2 was used
as standard. This solution was of the following composition:

Salts	Molarity
KH_2PO_4	0.0180
$Ca(NO_3)_2$	0.0052
$MgSO_4$	0.0150
Ferric tartrate	2 mgm.

(From 1 gm. of ferric tartrate dissolved in 1000 cc of
distilled water, 2 cc were taken.)

In addition, for the growth of the plants in the
experiment of Table 4, b) and c), the following solutions
were used:

	approximate osmotic pressure
b) 1/2 R5S2	.90
c) 1/4 R5S2	.48

Germination solution

When seeds were germinated in tap water, the change
of the seedlings from the tap water into complete culture
solution was too abrupt and caused injuries, so a dilute
culture solution was prepared by diluting one volume of 1/1
R5S2 with 4 volumes of distilled water.

One-salt solutions

For experiments in Tables 1, 2, and 3, one-salt solutions were used.

A stock solution of 0.5 moles of acid potassium phosphate in 1000 cc distilled water was prepared. From this solution the following aliquots were taken:

			molarity	gm. of K
For Tables 1 and 3,	a)	37 cc	0.0185	0.7233
For Table 1,	b)	18.5 cc	0.0093	0.3616
For Table 1,	c)	10 cc	0.005	0.1954

A stock solution of the same concentration was made up from calcium nitrate. The following aliquots were taken:

			molarity	gm. of Ca
For Table 2,	a)	22 cc	0.011	0.450
For Tables 2 and 3,	b)	11 cc	0.0055	0.225
For Table 2,	c)	5.5 cc	0.00275	0.1125

Osmotic pressure

The osmotic pressure is roughly proportional to the ionic concentration and can be calculated from thermo-dynamic data by the formula:

$$PV = nRT$$

where
PV = osmotic pressure - volume
n = ionic concentration
R = 0.08206, a constant - (liter atmosphere)
T = 273 (absolute temper) plus temperature of solution (42).

Taking of samples

Before samples were taken, either for chemical analysis or pH readings, the volume of culture solution was made up to 900 cc.

Methods of analysis

The following determinations were made:

Potassium -- Potassium was determined after the method developed by Volk, N. J. and Truog, E., "A rapid method for determining the readily available potash of the soil".

Calcium -- Calcium was determined after the method given out by the Bureau of Standards, "Determination of magnesium in Portland cement and similar materials by the use of 8-hydroxyquinoline".

Hydrogen-ion concentration -- The H-ion concentration was read from the Beckman pH-meter, manufactured by the National Technical Laboratories, Pasadena, California.

Germination and growth of plants

Hordeum vulgare was used in all experiments. The seeds were soaked in water for 10 to 15 hours (over night) and then placed upon a germination net which consisted of ordinary mosquito netting that had been dipped quickly into melted paraffin of a high melting point, so that the thread-work of the network was covered but the meshes remained open. The treated netting was tied as tightly as possible over the top of a Coplin jar and filled with germination solution so

that the surface of the solution was on the same level as the netting. The seeds thus came in contact with the solution and were kept moist constantly. The solution in the germination vessel was changed frequently by adding fresh solution through a glass tube that extended to the bottom of the container.

The temperature was controlled as much as possible. The germination jars were placed into diffused daylight and kept there until the seedlings had reached the desired stage of growth (6 to 8 cm). The seedlings were then selected while still over the germination solution, and carefully removed with paraffined forceps and placed into a large Petri dish containing a somewhat stronger germination solution (1 volume R5S2 to 2 volumes of distilled water). After a thorough washing they were placed in the culture jars.

As culture jars, ordinary Mason fruit jars of one quart capacity were used. The plants were held in flat 1/2 inch corks lightly covered with paraffin. Six young plants were placed in position in the holes of the cork and held firmly just above the seeds by means of non-absorbent cotton, which became compressed as the culms enlarged.

The culture jars were filled with 900 cc each of culture solution. The surface of the solution was within

1 to 2 cm of the loosely fitting corks. Cylinders of sheet
metal were placed around the jars to keep roots completely
in the dark. The plants were kept as nearly as possible
under uniform atmospheric condition (greenhouse). The
solution was changed twice a week. Clean jars were taken
every time. The vessels that had been used were emptied,
cleaned, and then refilled at the next change.

Aeration of solutions was found not to be necessary
since the solutions were changed at frequent intervals. The
oxygen supply in the fresh solution is sufficient for optimum
plant development for 3 days (L. H. Jones, Department of
Botany, Massachusetts State College). At the end of 18 days
the plants were thoroughly rinsed with distilled water for 5
minutes and then placed into the various media for absorption
and excretion studies.

For the study with excised roots, plants grown in
sand cultures were used. Since a well developed root system
was desired for this particular experiment, it was advisable
to use sand cultures in which the roots showed a more pro-
fused branching than roots developed in water cultures. The
plants received frequent applications of complete nutrient
solution (R5S2).

IV PRESENTATION OF RESULTS

The results obtained in this work are presented
in Tables 1 to 4 and Graphs 1 to 4. The values obtained are
always expressed in grams per 1000 cc of solution, showing
either an increase or a decrease of the elements in question
within the solution.

Table 1, wheat plants put into solution of acid potassium
phosphate of varying concentration (a-0.0185,
b - 0.0093, c - 0.005 molar).

Table 2, whole plants put into solutions of calcium nitrate
of varying concentration (a - 0.0110, b - 0.0055,
c - 0.00275).

Table 3, excised roots put into: 2) distilled water;
b) 0.0185 molar KH_2PO_4 solution; c) 0.0055 molar
$Ca(NO_3)_2$ solution.

Table 4, whole plants grown in: a) 1/1, b) 1/2, and
c) 1/4 R5S2 were put into distilled water.

All solutions were analyzed for Ca and K. The values are
expressed in gm/1000 cc.

The theoretical amounts of K and Ca in the solu-
tions were not obtained in the analyses, for example:

Theoretical: 37 cc of 0.5 M KH_2PO_4 in 1000 cc distilled
water equals 0.7233 gm. of K.

Analysis: 0.7038 gm. of K.

The difference of 0.0185 gm. was the mean.

Theoretical: 11 cc of 0.5 M $Ca(NO_3)_2$ in 1000 cc distilled

                   water equals 0.225 gm. of Ca.

Analysis: 0.208 gm. of Ca.

The difference of 0.017 gm. was the mean.

          These differences decreased nearly proportionally

with a decrease in concentration.

TABLE 1.

DETERMINATION OF K, Ca, AND pH IN SOLUTION AT DIFFERENT
TIME INTERVALS OF PLANT DEVELOPMENT

(Analyses expressed in gm/1000 cc)

Concentration of Medium	Initial Values			10 hours			24 hours			48 hours			72 hours		
	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K
a) KH$_2$PO$_4$, 0.0185 molar	5.01	--	0.7038	5.05	0.0018	0.703	5.16	0.0022	0.7014	5.23	0.0039	0.6817	5.25	0.0091	0.6798
				5.1	0.004	0.700	5.15	0.006	0.7009	5.29	0.009	0.6940	5.35	0.0071	0.6814
							5.2	0.0031	0.6912	5.3	0.0062	0.6910	5.38	0.0085	0.6830
							5.18	0.0042	0.6992	5.28	0.0078	0.6785	5.5	0.0189	0.6530
										5.33	0.006	0.6900	5.42	0.0102	0.6672
										5.35	0.008	0.6810	5.4	0.0091	0.6778
													5.41	0.0099	0.6730
													5.46	0.0123	0.6690
b) KH$_2$PO$_4$, 0.0093 molar	5.45	--	0.3519	5.5	0.003	0.350	5.61	0.009	0.3472	5.63	0.0097	0.3411	5.63	0.0093	0.3352
				5.6	0.007	0.3451	5.66	0.0097	0.3411	5.7	0.0109	0.3398	5.70	0.0112	0.3314
							5.7	0.0107	0.3456	5.71	0.0109	0.3918	5.68	0.0101	0.3118
							5.6	0.0097	0.3325	5.68	0.0100	0.3315	5.71	0.0119	0.3213
										5.7	0.0110	0.3275	5.75	0.0136	0.3181
										5.69	0.0102	0.3299	5.73	0.0145	0.3101
													5.8	0.0168	0.3253
													5.9	0.0195	0.3176
													5.43	0.0097	0.3385
c) KH$_2$PO$_4$, 0.005 molar	5.92	--	0.1759	6.1	0.009	0.1750	6.4	0.013	0.168	6.4	0.019	0.159	6.35	0.023	0.148
				6.3	0.012	0.1690	6.5	0.019	0.151	6.45	0.025	0.142	6.5	0.031	0.124
							6.35	0.011	0.1675	6.45	0.021	0.1556	6.43	0.041	0.140
							6.34	0.018	0.1346	6.4	0.27	0.128	6.41	0.037	0.1275
										6.39	0.0195	0.1465	6.46	0.029	0.1409
										6.42	0.022	0.1316	6.47	0.031	0.1297
													6.9	0.051	0.1388
													6.8	0.048	0.1307

Graph 1

GRAPHIC EXPRESSION OF RESULTS SHOWN IN TABLE 1

(Analyses expressed in mgm. per 1000 cc)
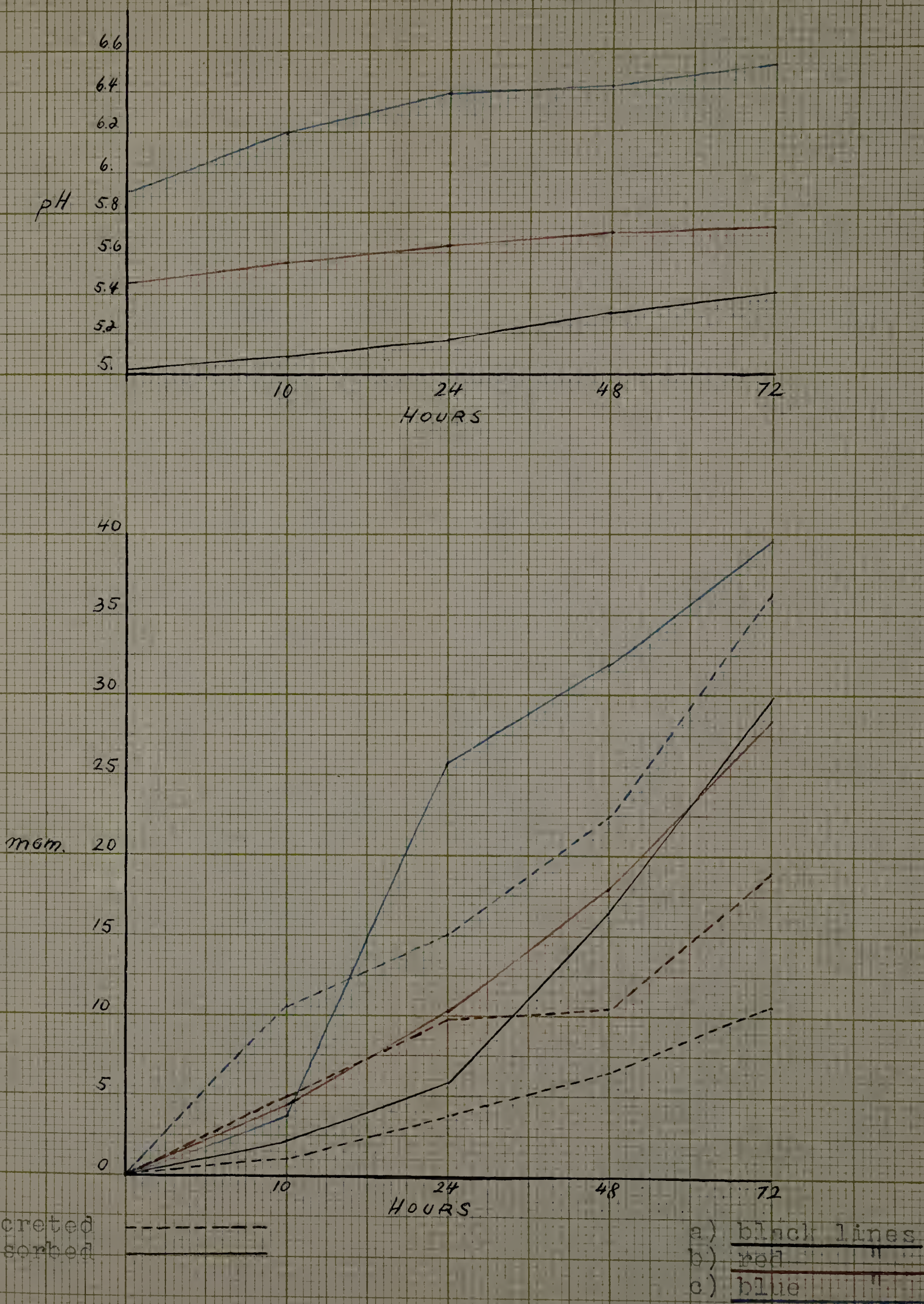
Ca excreted ------
K absorbed _____

a) black lines
b) red   "
c) blue   "

Table 1

Interpretation of results

The absorption of potassium increases absolutely over the period of three days. Still stronger than the absorption of potassium is the uptake of the phosphate ion shown by decreasing H-ion concentration. From the flattening out of the pH curve in b) and c), the writer concludes that the non-proportional absorption of cations and anions might slowly become more and more proportional, or that an increasing excretion of cations may take place. Small amounts of bicarbonates derived from the roots undoubtedly play an important part in the lowering of the H-ion concentration.

Since all plants, during the time before the experiment, were grown in complete culture solution, the accumulation of ions (if such an accumulation takes place) and the pressure within the root cells, were assumed to be approximately the same. The amounts and intensities of excretions varied directly with the osmotic pressure of the solutions. The least excretion was observed to be into the solution of highest concentration, and the strongest excretion into the solution of lowest concentration. In a) and b), excretion exceeded absorption during the first hours, which can entirely be ascribed to exosmosis, a

movement of ions from the roots into the medium.

In the case of absorption, one would expect to obtain the highest amounts from the strongest solution, but contrary to the expectation, 33 per cent more potassium was taken from the lowest concentration than from the highest. Thus, absorption was highest where excretion was highest. The lowest absorption and excretion occurred in the strongest solution (a).

This leads to the assumption, that as a healthy plant absorbs larger amounts of ions, more ions are given off. The changes in pH are characteristic of a greater uptake of anions than of cations (a and b). The strong decline of H-ion concentration in c is believed to be caused by larger amounts of bicarbonates, derived from the roots, than appeared in a and b (more intensive excretion). Salt concentrations for optimum development are far below the present standard concentrations of culture solutions.
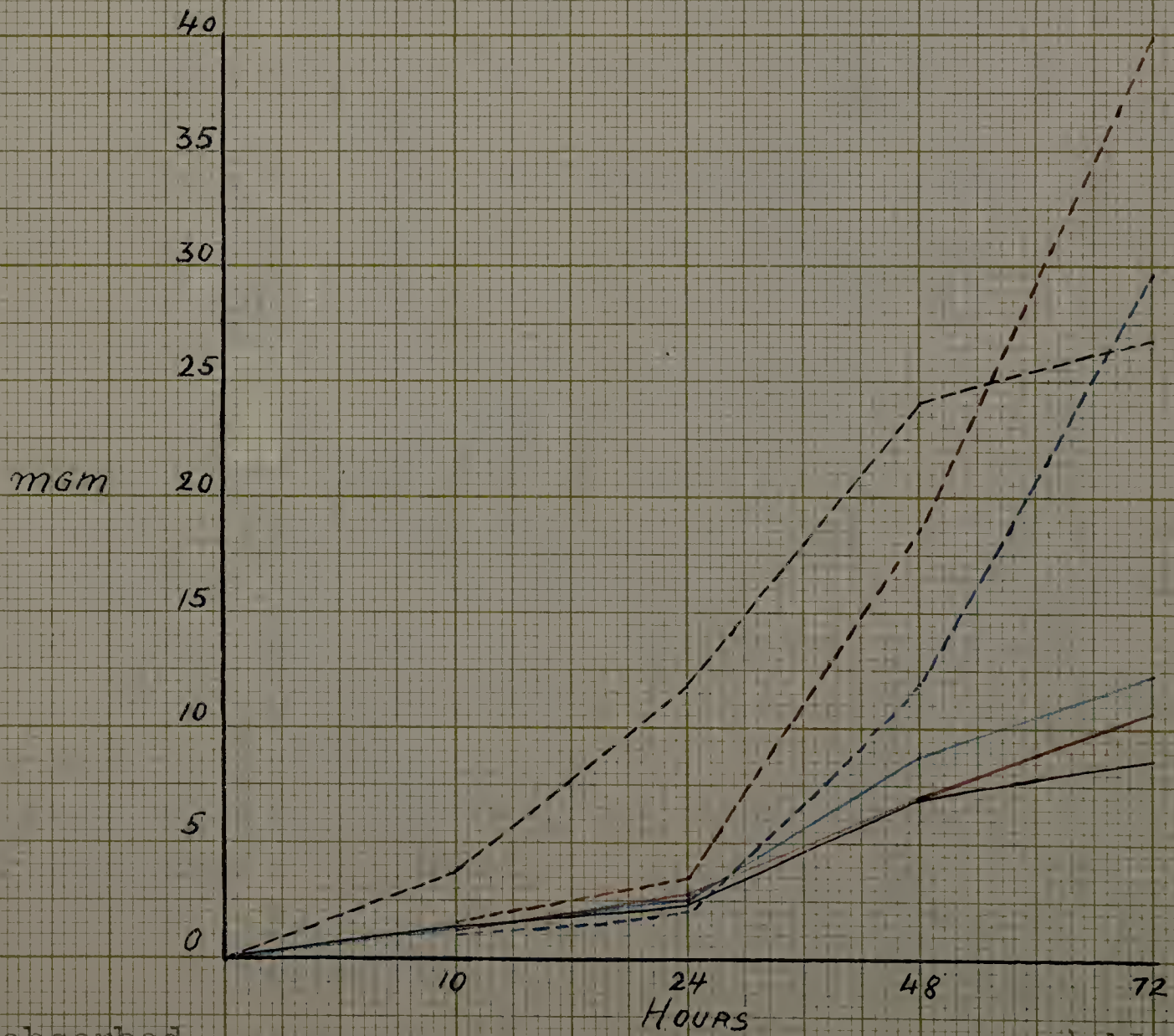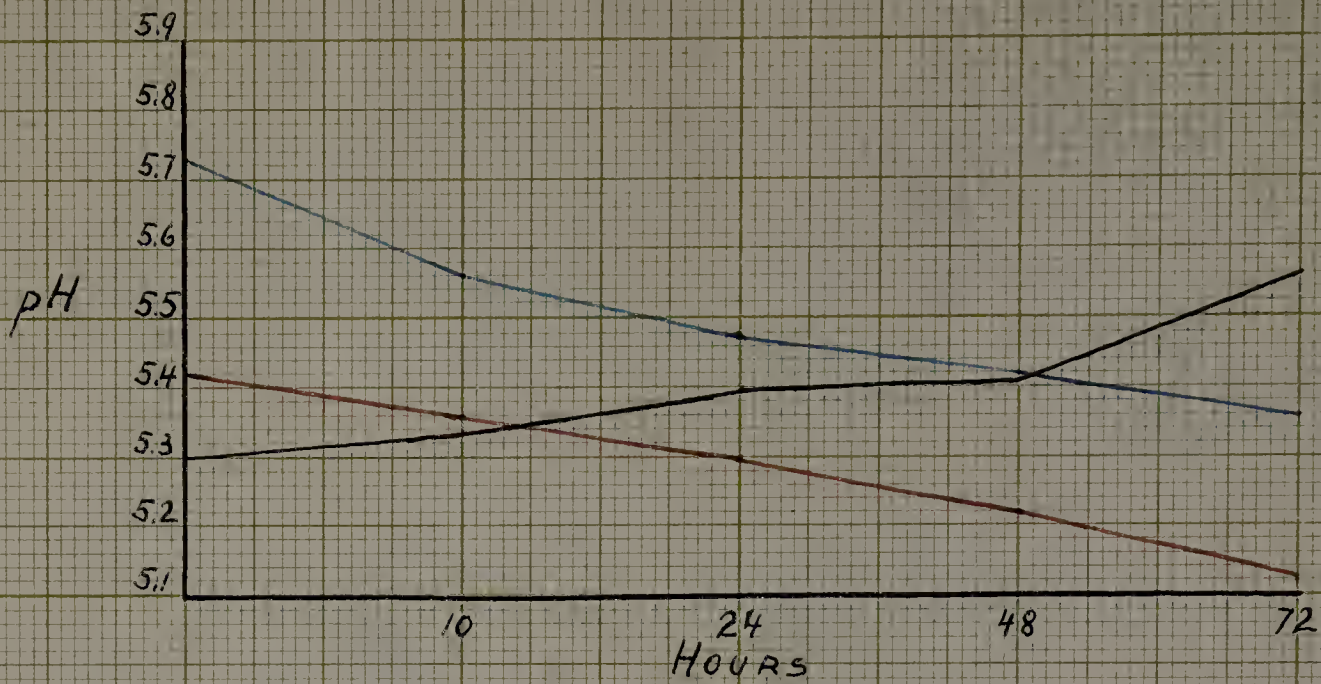
TABLE 2.

DETERMINATION OF K, Ca, AND pH IN SOLUTION AT DIFFERENT TIME INTERVALS OF PLANT DEVELOPMENT

(Analyses expressed in gm/1000 cc)

Concentration of Medium	Initial Values			10 hours			24 hours			48 hours			72 hours		
	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K
a) Ca(NO$_3$)$_2$, 0.0110 molar	5.31	0.416	---	5.3		0.0013	5.31	0.4102	0.0028	5.28	0.3932	0.0098	5.45	0.3822	0.0116
				5.33		0.0025	5.39	0.4037	0.0037	5.46	0.3907	0.0091	5.49	0.3499	0.0131
				5.36		0.0009	5.4	0.4039	0.0012	5.48	0.3911	0.0020	5.52	0.3800	0.0060
							5.51	0.4012	0.0021				5.71	0.3979	0.0087
							5.48	0.4029	0.0028				5.67	0.3876	0.0065
													5.51	0.3909	0.0052
													5.56	0.396	0.0097
b) Ca(NO$_3$)$_2$, 0.0055 molar	5.42	0.208	---	5.4		0.0019	5.35	0.2051	0.0027	5.25	0.1923	0.0099	5.18	0.1631	0.0141
				5.34		0.0012	5.28	0.2048	0.0029	5.17	0.1894	0.0090	5.1	0.169	0.0103
				5.36		0.0013	5.3	0.2039	0.0023	5.23	0.186	0.0081	5.14	0.161	0.0107
							5.25	0.2063	0.0024				5.08	0.166	0.0096
							5.31	0.2021	0.0038				5.11	0.1727	0.0105
													5.17	0.1819	0.0097
													5.06	0.163	0.0102
c) Ca(NO$_3$)$_2$, 0.00275 molar	5.73	0.104	---	5.4		0.0019	5.55	0.101	0.0026	5.51	0.0984	0.0081	5.49	0.0817	0.0132
				5.64		0.0016	5.61	0.102	0.0024	5.48	0.0962	0.0099	5.5	0.0796	0.0106
				5.55		0.0012	5.51	0.101	0.0031	5.50	0.089	0.0089	5.45	0.0710	0.0143
							5.4	0.1014	0.0035	5.31	0.0860	0.0086	5.3	0.0685	0.0111
							5.35	0.100	0.0029	5.32	0.0901	0.0091	5.25	0.0610	0.0106
													5.41	0.075	0.0148
													5.12	0.0812	0.0121

Graph 2

GRAPHIC EXPRESSION OF RESULTS SHOWN IN TABLE 2

(Analyses expressed in mgm. per 1000 cc)

Ca absorbed   ------------
K excreted   ——————

a) black line
b) red
c) blue

Table 2

Interpretation of results

The absorption of calcium increases with the length of the experimental period. The nitrate ions are taken up in larger amounts and hence a decrease in H-ion concentration. The alkalinization by nitrate ion is a well known occurrence and was to be expected.

Here again the absorption greatly exceeds the excretion. The solution with highest concentration of calcium nitrate (a) showed a markedly less exchange (absorption and excretion) than c and b. Solution b, with half the strength of a, shows the highest exchange values, followed by c which was the solution of lowest osmotic pressure with the highest amount of excretion. The weakest salt solution, however, did not result in highest absorption and excretion values as in Table 1, which according to Johnston and Hoagland (34) is due to the necessity for higher concentration of calcium ions than of any other cation for optimum development. The low osmotic pressure in solution c caused an excretion for the first 24 hours, higher than the absorption (exosmosis).

In b and c a considerable increase in H-ion concentration occurred. This is explained by Niklewski,

Krause, and Lemanczyk (35) who stated that in low calcium nitrate concentrations calcium is taken up in far larger amounts than NO_3. A lowering of the pH of the solution to 4.4 results in an equivalent uptake of Ca-ions and NO_3-ions; and at pH of 5.3 twice as many Ca as NO_3 ions are absorbed. At the isoelectric point, pH 4, the absorption of Ca and NO_3 ions will be equal (35).

A relationship concerning a proportional exchange of potassium and calcium (K for Ca and vice versa) in either one of the two experiments could not be found.
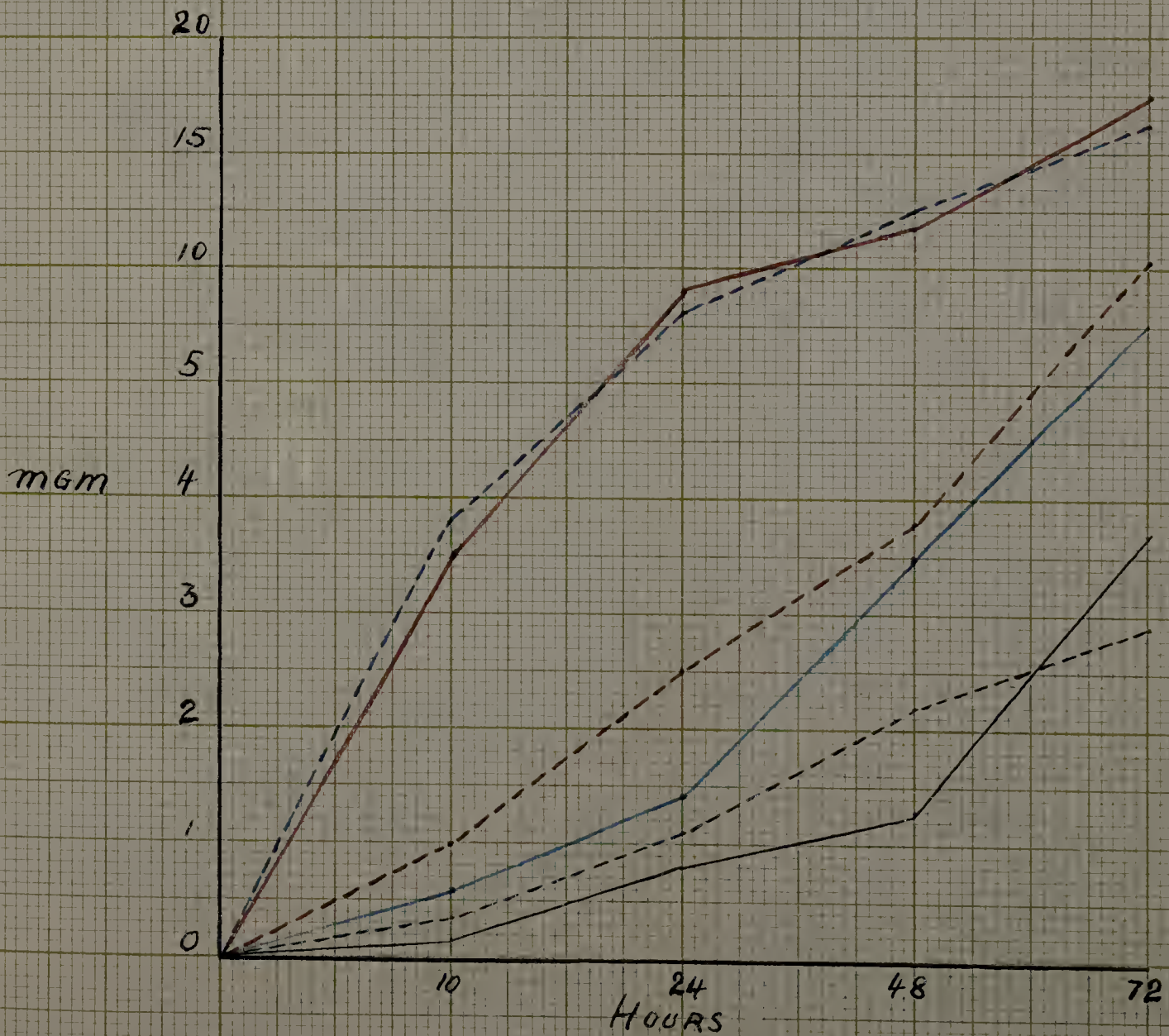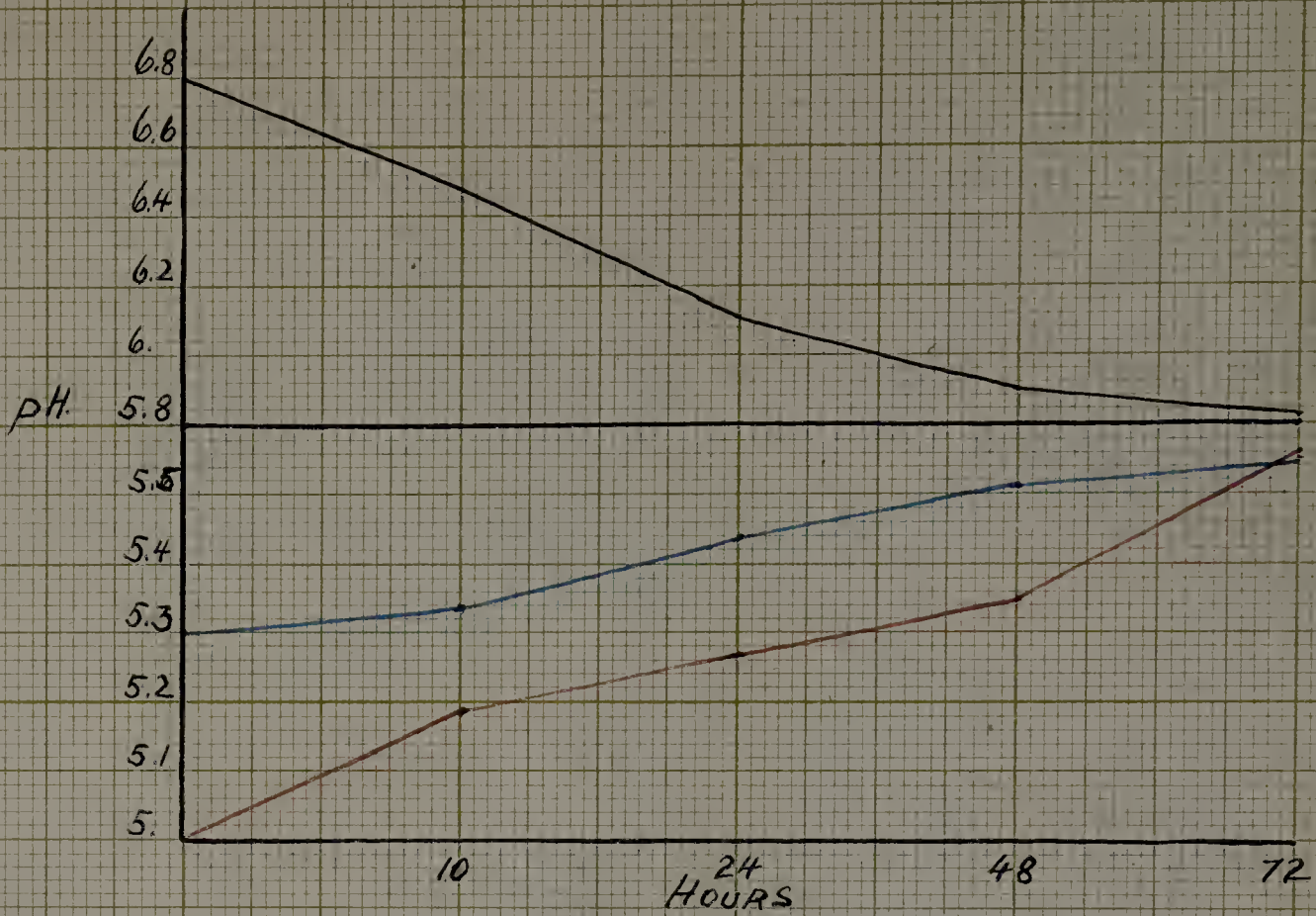
TABLE 3.

DETERMINATION OF K, Ca, AND pH IN a) DISTILLED WATER,
b) KH_2PO_4, c) $Ca(NO_3)_2$ AT DIFFERENT INTERVALS OF
CONTACT WITH EXCISED ROOTS

(Analyses expressed in gm/1000 cc)

Medium of excised roots	Initial Values			10 hours			24 hours			48 hours			72 hours		
	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K
a) Distilled Water	6.8	--	--	6.51	None	Trace	6.2	0.0009	0.0006	6.0	0.0015	0.0012	5.9	0.0022	0.0051
				6.42	Trace	None	6.0	0.0013	0.00085	5.8	0.0028	0.0011	5.75	0.0031	0.0037
				6.48	Trace	None	6.15	0.0011	0.0009	5.91	0.0023	0.0016	5.8	0.0036	0.0031
b) KH_2PO_4, 0.0185 molar	5.01		0.7038	5.1	Trace	0.7001	5.18	0.0016	0.6978	5.31	0.0029	0.6912	5.42	0.0097	0.6871
				5.25	0.0011		5.36	0.0032	0.6952	5.4	0.0048	0.6914	5.51	0.0101	0.6860
				5.22	0.0009		5.34	0.0027	0.6967	5.45	0.0052	0.6881	5.6	0.0098	0.6801
				5.12	Trace		5.15	0.0011	0.6991	5.22	0.0022	0.6972	5.7	0.0112	0.6898
c) $Ca(NO_3)_2$, 0.0055 molar	5.3	0.208	--	5.35	0.2041	None	5.42	0.201	0.0016	5.43	0.1981	0.0039	5.5	0.1960	0.0059
				5.4		Trace	5.51	0.1989	0.0021	5.59	0.1961	0.0041	5.6	0.1940	0.0067
				5.32			5.4	0.1994	0.0011	5.45	0.1972	0.0029	5.51	0.1953	0.0053
				5.3			5.44	0.1998	0.0009	5.55	0.1905	0.0032	5.62	0.1818	0.0119

Graph 3

GRAPHIC EXPRESSION OF RESULTS SHOWN IN TABLE 3

(Analyses expressed in mgm. per 1000 cc)

a) $\dfrac{Ca \text{ excreted}}{K \text{ excreted}}$ b) $\dfrac{Ca \text{ excreted}}{K \text{ absorbed}}$ c) $\dfrac{Ca \text{ absorbed}}{K \text{ excreted}}$

Table 3

Interpretation of results

a) <u>Distilled water</u>. The appearance of calcium and potassium in distilled water was caused by a natural function of the roots. As previously mentioned, roots are believed to accumulate salts (in storage tissues of the roots) which the plant is unable to retain against the rinsing effects of water. The amounts given off are small compared to those in Tables 1 and 2, where it is believed that an ionic exchange took place. Both Ca and K are given off in approximately the same amounts. The retentive power of the root is higher for K than for Ca during the first 2 days; then, however, K is lost in larger quantities than Ca. It is assumed that Ca is present in larger quantities than K which explains the quicker appearance of the former. That excreted cations are largely responsible for the increase in H-ion concentration cannot be said safely, since many other materials (acids, enzymes, organic substances) are given off, particularly into distilled water (36).

b) <u>Acid potassium phosphate</u>. The absorption of potassium by excised roots could be detected right from the beginning. It took place during the first 24 hours at a much faster rate than by the whole plants (see Graph of Table 1), but at the end of the experimental period of 72

hours reached only 17.45 mgm. as compared to 29.8 mgm. by the whole plants. The pressure of the root cells caused the appearance of droplets on the newly-cut surfaces, indicating that the roots tried to transport absorbed ions to the upper organs.

The amount of calcium excreted nearly reached that excreted by the whole plants. The writer assumes that the roots have a reserve of ions which are given off in order to restore as fast as possible a physio-chemical balance between the roots and the medium, after which a more or less ionic exchange may occur.

The strong decrease of H-ion concentration was probably due to a small potassium uptake and to a high absorption of phosphate ions. A general decrease in activity during the second day seems to affect all values. A remarkable flattening out of all the curves, particularly of the pH curve, indicates that the absorption of phosphate ions was considerably reduced.

c) <u>Calcium nitrate</u>. Calcium was absorbed by excised roots at a faster rate during the first 24 hours of the experiment than by the whole plants, amounting to 16.2 mgm. at the end of 72 hours as compared to 39.8 mgm. by the whole plant over the same period.

The excretion of potassium by the excised roots

was 29 per cent less than by the whole plants.   It was previously assumed that potassium ions are held more firmly by the roots than calcium, which is here again confirmed, for Ca (in b) was given off in almost equal amounts as by the whole plants.

The H-ion concentration decreased, which is explained by predominating absorption of NO_3-ions.
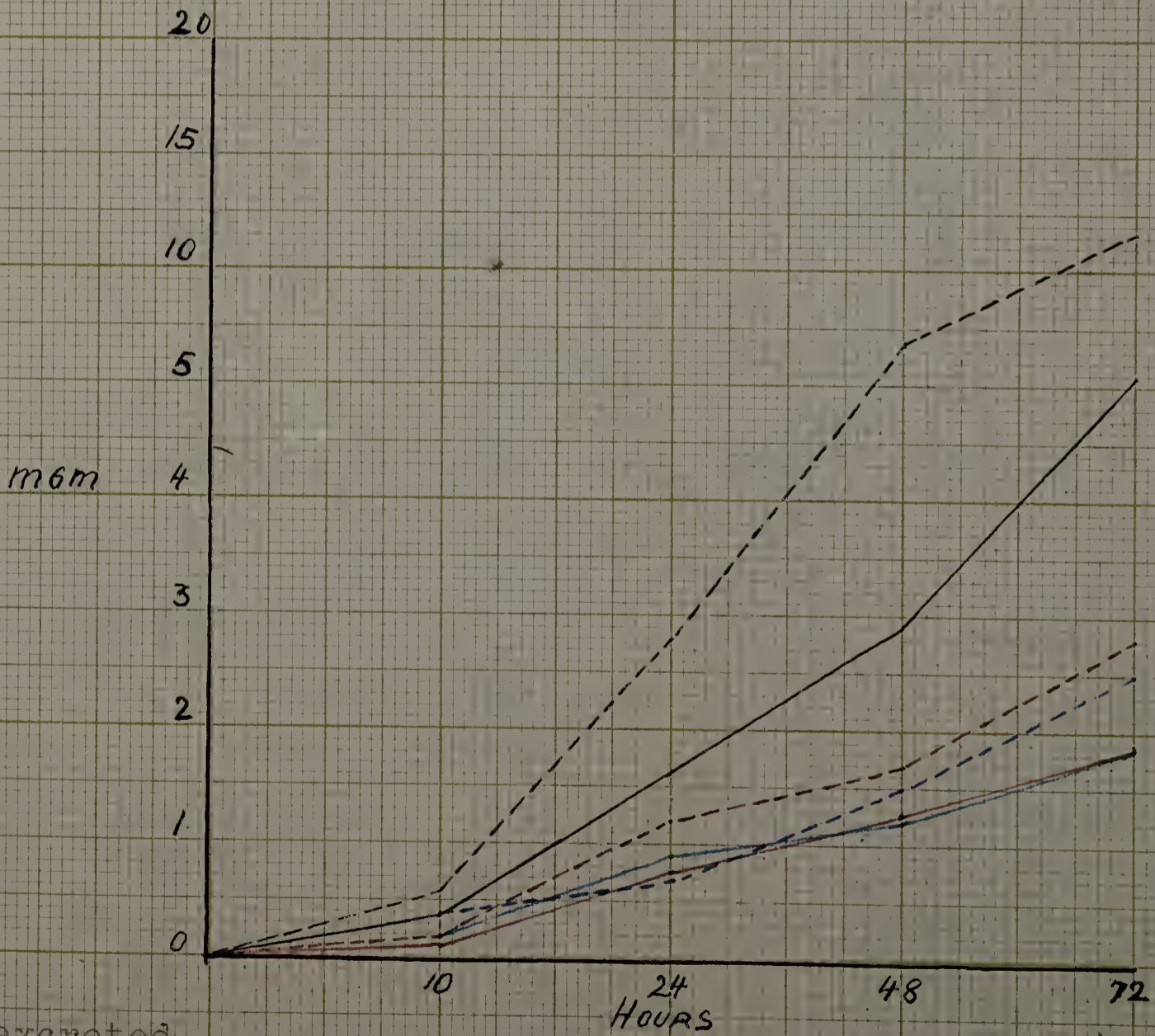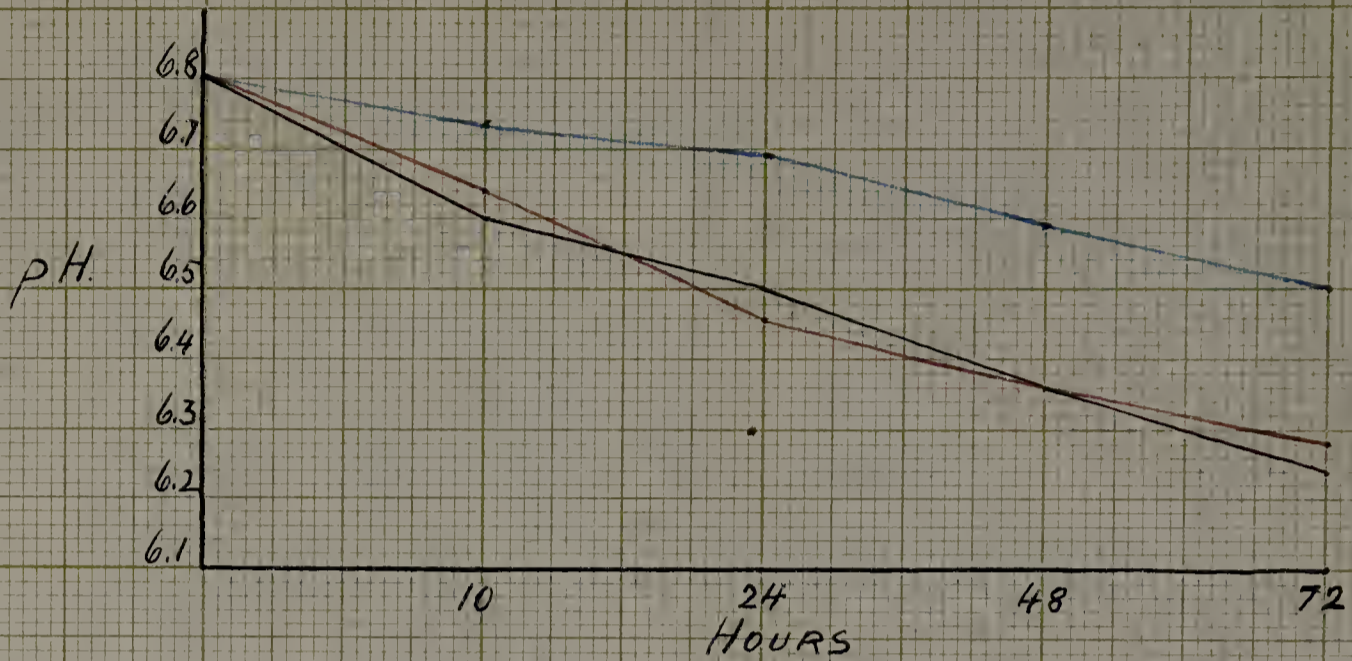
TABLE 4.

DETERMINATION OF K, Ca, AND pH IN DISTILLED WATER AT DIFFERENT TIME INTERVALS OF PLANT DEVELOPMENT

(Analyses expressed in gm/1000 cc)

Medium	Initial Values			10 hours			24 hours			48 hours			72 hours		
	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K	pH	Ca	K
a) Distilled water	6.8	--	--	6.7	Trace	None	6.7	0.0012	0.0006	6.5	0.0051	0.0014	6.35	0.0089	0.003
	6.8			6.5	0.0010	0.0005	6.4	0.0025	0.0014	6.35	0.0067	0.0020	6.21	0.0098	0.0045
	6.8			6.55	0.0009	0.0009	6.33	0.0040	0.0023	6.39	0.0079	0.0031	6.18	0.0103	0.0067
	6.9			6.45	0.0013	0.0012	6.4	0.0056	0.0034	6.2	0.0082	0.0065	6.07	0.0200	0.0097
	6.8			6.75	Trace	Trace	6.68	0.0010	0.0009	6.53	0.0062	0.0021	6.4	0.0092	0.0029
b) Distilled water	6.8	--	--	6.76	None	Trace	6.51	0.001	0.0008	6.4	0.0015	0.0012	6.35	0.0026	0.0016
				6.71	Trace	Trace	6.59	0.0015	0.00065	6.38	0.0017	0.0011	6.3	0.0024	0.0019
				6.76	None	None	6.45	0.0017	0.0009	6.38	0.0019	0.0014	6.24	0.003	0.002
				6.54	Trace	None	6.41	0.0009	0.001	6.40	0.0016	0.0017	6.33	0.0029	0.0025
				6.49	Trace	Trace	6.38	0.0011	0.0005	6.35	0.0020	0.001	6.21	0.0031	0.0015
c) Distilled water	6.9	--	--	6.8	Trace	Trace	6.75	0.0009	0.0007	6.55	0.0013	0.0010	6.5	0.0036	0.0019
	6.8			6.73	Trace	Trace	6.68	0.0010	0.0008	6.61	0.0016	0.0011	6.51	0.0028	0.002
				6.76	0.0009	0.0007	6.71	0.0013	0.0010	6.64	0.0015	0.0016	6.53	0.0019	0.0021
	6.7			6.68	0.004	Trace	6.63	0.0008	0.0011	6.58	0.0012	0.0012	6.51	0.0017	0.0019

Graph 4   35.

GRAPHIC EXPRESSION OF RESULTS SHOWN IN TABLE 4

(Analyses expressed in mgm. per 1000 cc)

Ca excreted
K   "
                                    a) black lines
                                    b) red   "
                                    c) blue   "

Table 4

Interpretation of results

      The growth of the plants in different nutrient - solution concentrations (R5S2, a - 1/1; b - 1/2; c - 1/4) accounted for the varying amounts of calcium and potassium excreted. This leads to the belief that, since all the plants developed well in each concentration, that plants have a distinct ability to accumulate ions. That such an accumulation has some significance and probably is of special importance to the plants must be assumed. The accumulation might serve as a storage of energy which continually is used in an exchange for other ions (in distilled water, very likely H- and OH-ions).

      This experiment again proves that potassium is more tightly held by the roots than calcium. The excretion of calcium was higher in the more acid media. A great acceleration was observed around a pH of 6.5. The explanation for minimum excretion at high pH is, according to Tueva (12), that the salts within the plants are in an isoelectric condition.

Conclusion: The lower the pH of the medium, the higher the excretion of calcium; the point of greatest acceleration is around pH 6.5

      Plants (barley) are able to take up more potassium and calcium than is absolutely necessary for normal development.

V DISCUSSION

Just how the differences in results, between some experiments of previously mentioned investigations and this work, are to be accounted for, cannot be explained. Possibly the experimental conditions, such as species of plant, time and length of experiments, treatment of plants, salt concentrations, etc., had a great influence. It is generally known that a repetition of a physiological experiment is more difficult the less one knows about the factors influencing it decisively.

In this work the conditions were of such nature that an equivalent exchange of Ca and K could not be accounted for. Under different conditions completely new results might be obtained. The question, how these results can be explained, must remain open. However, the following should first be considered. With the growth of a plant a necessary uptake and accumulation of elements has to take place. A constant exchange of cations for cations, and anions for anions, would result in a cycle which would not explain the increase in ash substances. Therefore, the activities of H-ions and OH-ions, and further bicarbonate ions, must necessarily be considered. The effects of H- and OH-ions have previously been mentioned. The bicarbonate ions, which come indirectly

from the atmosphere, or are freed by the respiration of carbohydrates and decarboxylisation of organic acids, are available for the plants in large amounts. Further, an excretion of organic ions undoubtedly plays an important part in the cycle. That such excretions took place in this work showed the necessity for removal of organic compounds before potassium tests could be successfully concluded. (See, Methods of analysis). The amounts of nature and these organic materials were not determined.

In discussing in a more general way the results presented in this work, the writer concludes first, that the processes of absorption and excretions have to do with ions. The data obtained are in favor of this conclusion. Jenny (28) in recent works suggests that plants do not behave in soils as they do in cultures, in that they may absorb colloidal substances from the soil or at least a diffusion may occur through the intimate connection of root hairs and soil particles. While it may appear probable that absorption processes are concerned with ions, it certainly should not be assumed that ions act as completely independent units.

On the contrary, it is very well possible, and clearly understandable, that one ion influences the absorption of another ion. It does not appear, however, that it is wise to interpret the results of culture-solution experiments

in terms of salt proportions. In solutions of the dilution
with which the writer is concerned, a high percentage of
dissociation must exist. The actual analysis of such solu-
tions must be made on the basis of ions or of chemical
elements, and it is obvious that the results of absorption
studies can best be expressed in these terms. Moreover,
the interpretations of the data must almost necessarily be
made in terms of ions or chemical elements.

Pantanelli (19) drew the general conclusion, from
his absorption studies, that the two ions of a salt are
seldom removed from solutions in equal proportions, which
conclusion is, on the whole, in agreement with the data and
assumption of this work.

If there is extensive inequality in the absorption
of positively and negatively charged ions, an exchange of
one ion for another must take place, since the change of
H-ion concentration, which can occur, is comparatively
limited. The reason why nitrate ions are absorbed in far
greater amounts than most cations (Ca probably is the
exception) is the ease of a replacement of nitrate ions by
bicarbonate ions. In experiments represented in Table 2,
c and b, such a replacement apparently did not take place,
since this would result in a decrease of H-ion concentration.

In earlier works (15, 18), the view was held, that

positive and negative ions are either absorbed in equal
proportion, or else that an exchange of ions occurs. In
the light of present knowledge, however, it is difficult to
say whether, for example, potassium may be absorbed from
acid potassium phosphate as potassium hydroxide, or whether
the potassium ion may replace the H-ion directly to the
limited extent that corresponds to the increase of H-ion
concentration taking place in the solution.

That the permeability of the root tissues is of
utmost importance is clear. True (38) emphasizes the
importance of calcium in preserving the normal permeability
of plant cells. The rate of penetration of the ions is
perhaps closely connected with this. Why are some ions
absorbed much more slowly than others? It is quite apparent
that not only the chemical characteristics of the ion, but
also those of the cell membrane, must be taken into con-
sideration.

The intensity of ionic absorption was, in all
experiments, directly related to the osmotic pressure of
the solution. It is of particular interest to see that
potassium was absorbed at a faster rate than calcium. For
example, during the first ten hours, for all absorption
experiments, 10.3 mgm. of potassium, against 6.5 mgm. of
calcium, were absorbed. This result agrees very well with

observations by Wieringa (31), than an ion is taken up more easily the farther to the left it is in the lyotropic ion column. This result, however, cannot be considered as being definite, since the experiments were not conducted at the same time. In addition, it should be mentioned that the amount of ions absorbed increased as the time went on (within the period of experiment). The proportions of anions to cations absorbed changed continuously, with the anions predominating in the long run. This was confirmed by a steady decrease of H-ion concentration until the ninth day, and then a sudden rise, followed by a decrease again. This interruption could be observed in all culture solutions and agrees with Shive's (39) observations. According to Redfern (23), ions are absorbed until the plasma colloids are saturated, followed by a resting period, after which ions again are absorbed.

Experiments conducted with excised roots gave results similar to whole plants; that is, the amount of total potassium or calcium absorbed by the roots was less than by the whole plant, and the amount of potassium and calcium given off by roots was about the same as by the whole plant. This behavior of excised roots leads to the assumption, that an absorption of ions by the whole plant is not only an absorption process by the root tissue, but that the upper

organs play an important role. However, it was stated many times, that the absorption of salts is entirely independent from the amount of water taken up, and that it depends principally on the intensity of growth (32, 33). In this work, however, it was found that roots alone are not capable of a uniform ion uptake. This might be ascribed to the absence of the other organs to which salts would be trans-ported, or a non-occurring transpiration and consequently no suction action upon the root cells. Root pressure alone does not seem to be sufficient, since small droplets appeared on the newly-cut surface, which were renewed readily when wiped off.

It is clear that roots alone are not suitable for a study of ionic absorption. The upper organs apparently, on account of their transpiration, play an important part in the uptake of salts.

In the experiments reported in this paper, very brief periods of absorption and excretion were considered; but even in these brief periods, growth and metabolism were undoubtedly active, and the absorption of ions which occurred during the periods in question cannot be dissociated from these processes. The point of view which it is sought to suggest here is entirely different from that which assumes any type of chemical equilibrium between the plant and its

culture medium. It cannot be doubted that the plant cell operates in accordance with the laws of chemistry and physics, but the system is a dynamic one, and the energy relations of the chemical reactions taking place are, as yet, unknown.

Finally, it must be very emphatically stated, that the absorption of ions by a plant depends not only upon the culture solution, but also upon condition of light, temperature, and humidity, and therefore it cannot be expected that two experiments will give identical results, unless all conditions are the same. The experiments described in this work are useful only in so far as they throw some light on the general principles of ion absorption and excretion, and the consequent changes in H-ion concentration.

VI SUMMARY AND CONCLUSIONS

1. Determinations were made of the absorption and excretion of potassium and calcium, by (barley) plants and excised root systems of barley, from and into one-salt solutions of different concentrations, and into distilled water. A study of the changes in Hydrogen-ion concentration was also included. The salts used were acid potassium phosphate (KH_2PO_4), and calcium nitrate ($Ca(NO_3)_2$). The length of the experimental periods was 72 hours for all sets.

2. The intensity of absorption and excretion increased with the length of the experimental period. The absorption increased in general rapidly after the first 24 hours, while the excretion increased slowly throughout.

3. The reaction of the media was never stable in the presence of live root systems. The pH values increased during the daylight hours and decreased somewhat during the night.

4. The continual change of pH values was, undoubtedly, tied up with absorption and excretion phenomena of electrolytes, but to state that the degree of change was absolutely proportional to the rates of absorption and excretion would imply the exclusion of buffer action and other controlling factors.

5. From the total quantity of absorbable ions present, the proportion absorbed during a given period decreased as the concentration of these ions in the solution decreased. In this way plants can adapt themselves, to a considerable extent, to solutions of low concentrations.

6. For the first two days potassium was more firmly held by the roots than calcium, after which calcium appeared in larger amounts.

7. An equivalent absorption and excretion of calcium and potassium did in no instance take place. For extremely short periods only equivalent amounts could be detected.

8. A considerable excretion of potassium and calcium was not observed.

9. The results with excised roots show that roots alone are not capable of a uniform absorption of ions.

10. The excretion of ions into salt solutions was greater than into distilled water.

11. Attention is called to the fact that energy exchanges are involved in the processes of absorption and excretion. Permeability and osmosis alone are inadequate to explain these phenomena in the living plant.

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