THE INFLUENCE OF SOME EXTERNAL FACTORS ON GROWTH AND PHOSPHATE UPTAKE OF MAIZE PLANTS OF DIFFERENT SALT CONDITIONS

by

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CHAPTER I.

INTRODUCTION.

When studying the literature concerning the manner in which ions are absorbed by plants, one is struck by the fact that the majority of the data have been obtained, not from experiments with complete higher plants, but rather from experiments with certain parts of plants. Usually each 'school' uses its own material; much used objects are the separate cells of large-celled Algae such as Chara, Nitella, and Valonia, (OSTERHOUT, BROOKS, COLLANDER, HOAGLAND), small slices of parenchymatic tissue of storage organs, (STEWARD, STILES, ROBERTSON), leaves of aquatic plants, (ROSENFELS, ARISZ) and excised root systems (HOAGLAND, LUNDEGÅRDH). The purpose of this method is clear: When one cell, or a collection of cells as nearly equivalent to each other as possible, is used, the material will behave as one whole i.e. the diverse factors influencing the absorption of salt will have the same influence on all the cells. Certain laws governing the process of absorption are found more quickly and clearly in this manner. Moreover, reproduceable results can be more easily obtained with a comparatively small quantity of material, which simplifies the experimental technique.

With an entire plant, on the other hand, the process is much more complicated, since the ions which are absorbed by the root are, at least in part, transported to the shoot, whereas conversely, the root with regard to the supply of the carbo-hydrates required for the process of absorption is dependent on the shoot. That this interaction between root and shoot has, indeed, a great influence on the absorption is proved by the experiments of HOAGLAND and BROYER (1936, 1943). Excised roots of barley plants are only capable of absorbing K and Br when they are in low-salt condition i.e. when they have a high percentage of carbohydrates and a low percentage of ions. This absorption, however, does not continue for long; in a few days it comes to a complete standstill. Roots in high-salt condition i.e. with a high percentage of salt and a low percentage of carbo-hydrates are not capable of absorption. A decrease of the absorption with low-salt roots is thought to be the result of the fact that, here, neither a supply of carbo-hydrates from the shoot is possible, nor a transport of ions to the shoot. If, however, the

absorption is determined with intact plants in high-salt and low-salt condition, then it becomes evident that with both kinds of material it can continue for a longer period. From this it follows that the absorption by the roots of barley plants is influenced by the shoot. The writers, however, assume that this influence isexercised through the root metabolism so that the activity of the root always remains determinative for what is absorbed by the whole plant. Also VAN DEN HONERT (1933) who has investigated the absorption of phosphate by sugar cane, found that the concentration of the phosphate ions and the temperature are determinative for what is absorbed by the plant. He did not find any influence of the shoot on the absorption by the root, and he assumes that the activity of the root is determinative for the quantity of ions entering the plant. Other investigators, on the other hand, are of opinion that the shoot has a more direct influence on the absorption by the root. Thus, SCHMIDT (1936) finds that the transpiration of the shoot and its exposure to light have a distinct influence on the absorption by the root. As the influence of light is not the same for all the ions, but is especially marked for the K ions SCHMIDT assumes that K plays a part in the photosynthesis and that therefore a more direct influence of light exists on the absorption by the root. LUTTKUS and BÖTTICHER find the K absorption strongly influenced by exposure to light of the shoot, and they, too, assume a more direct activity of light. From these investigators it therefore appears that with higher plants the shoot influences the absorption by the root, but as to the question whether this takes place through an influence on the root metabolism or whether there may be a more direct influence of the shoot, opinions differ.

I have attempted in this investigation to examine more precisely the connection between root and shoot as regards the absorption of phosphate and I have done so with both high-salt and low-salt plants. In the first place I have examined more closely the connection between absorption and growth. It is well known that an insufficient supply of certain ions results in diminished growth, but this relation has not been further determined and there is, in my opinion, a possibility that with an adequate supply of ions the growth of the shoot may influence the absorption by the root.

It has become evident from experiments by HOAGLAND and his co-workers that there is a considerable difference in absorption between low-salt and high-salt plants; I have used both kinds of material for this investigation, in order to try to reach a clearer insight into the processes of absorption by means of these differences. Along with this comparison between absorption and growth I

also examined the influence of some external factors on the absorption of phosphate, viz. the exposure to light, and the phosphate concentration of the solution and also the degree in which this influence differs for both kinds of material. In the course of the investigation into the influence of exposure to light, the growth as well as the absorption was determined, whilst along with the influence of the phosphate concentration I also examined the influence of the concentration of other anions on the phosphate absorption.

The investigation was carried out in the botanical laboratory of the State University of Groningen. It was commenced in 1943, interrupted on account of the war, and continued in 1946 and 1947. I am greatly indebted to Professor Dr W. H. ARISZ for his stimulating advice, and for his constructive criticism.

CHAPTER II.

MATERIAL AND METHOD.

§ 1. *The cultivation of the material.*

As experimental plants young maize plants were used; the method of cultivation is as follows:

Eighty seeds are soaked in tap-water for a space of two days, then washed a few times, also in tap water, and subsequently spread on a piece of wide meshed cloth stretched on a wire ring. This ring stands on three legs in a cylindrical glass vessel on the bottom of which is a layer of water about 1 cm. in depth. On the outside the vessel is pasted over with black paper. A glass plate, also covered with black paper, is put on top of this cylinder so that the seeds are shut off from the light, and the whole is placed in a damp and hot green-house.

In about a week's time each seed has developed a root — mostly without lateral roots — of about ten centimeters and a coleoptile of two to three cm. These plants are now joined together in groups of three, and of each group the coleoptiles are, by means of a piece of non-absorbent cotton wool, wedged into a hole of about one cm. in diameter, which is bored in a wooden disc of twelve cm. diameter. Each disc has five holes, but only two of them are filled with plants, so that there are always six maize plants to each disc. Of the other holes one serves as a passage for the air tube necessary for the aera-

tion of the solution, the two others are meant as openings for the supply and outlet tubes in case of a continuous flow.

These discs are now placed on the top of glass jars of about one and a half liter capacity filled with nutrient solution. The jars are enclosed in a casing of corrugated cardboard in order to avoid the growth of algae as well as too great fluctuations of temperature.

During the summer months (April - November) the young plants were further cultivated in a green-house where, on account of the high temperature and the low humidity, they grow extremely well, so that in about four weeks they can be used for the experiment. The shoots are then about 30 cm. high, the root system shows abundant ramifications and fills a large portion of the culture vessel. During the other months the plants were cultivated in a smaller heated green-house but the growth is then considerably less. They develop into long light-green plants with a rather weakly developed root system. All the experiments mentioned in connection with the research were made with summer material; the winter material was used for orientation tests, which consequently were later on repeated with summer material when definite indications had been obtained.

As nutrient solution that of HOAGLAND and BROYER (1936) was used, but only of half strength, since the plants thrive better on this. The mixture used was as follows:

KNO_3	0,00125	mol
$Ca(NO_3)_2$	0,00125	,,
$MgSO_4$	0,00050	,,
KH_2PO_4	0,00025	,,

It was prepared with stock solutions of a twohundred fold concentration. At first 1 c.c. of saturated iron tartrate solution and 1 c.c. of the Hoagland A-Z solution (see SCHROPP and SCHARRER 1933) were added. It appeared, however, that plants cultivated on this solution always became chlorotic, so that extra iron had continually to be added. In most cases, however, the chlorosis could not be sufficiently combated in this way. Accidentally it was discovered that plants grown without phosphate did not become chlorotic and that, moreover, in this case, the very slight opalescence otherwise shown by the solution was absent. An attempt was made to remove these symptoms of deficiency of iron by using iron humate instead of iron tartrate. According to BURK, LINEWEAVER and HORNER (1932) humic acids have proved to be suitable for keeping iron in a state of solution in a complex compound. The iron humate was prepared according to VAN DEN HONERT (1933). As a matter of fact,

however, the results of my experiments did not differ from those with iron tartrate solutions, and therefore for the sake of convenience the latter were nevertheless used. However, in this case it was not directly added to the solution. At first the young plants remain perfectly green, even without iron, and grow quickly until in about two week's time the new leaves begin to show a lighter green. The nutrient solution is then drawn off by means of a syphon and replaced by a solution without phosphate but with iron tartrate. Evidently the plants had been able to take up sufficient phosphate already during the first period, for symptoms of deficiency of phosphor (purple-coloured leaf margins) did not occur in this way. The same method was also used by PARKER (1927).

In this solution, which is occasionally replenished with tapwater, the plants are further cultivated. This replenishment must take place twice a week in the middle of the summer, and once a fortnight in winter until the plants are used for the experiment. We have then the disposal of useful green plants, with a comparatively low salt content, with which a strong absorption may be obtained.

The age at which the plants are used depends upon the season. In winter they grow considerably slower than in summer, and the time of cultivation is consequently much longer. In midwinter it amounts to eighty or ninety days, in spring, summer and autumn from twenty to forty days.

§ 2. *The obtaining of low-salt and high-salt plants.*

The terms "low-salt" and "high-salt" were first used by HOAGLAND and BROYER (1936). By cultivating young barley plants on a nutrient solution, which was in the one case never, and in the other repeatedly renewed, they obtained in the first case plants with roots poor in salts and rich in carbo-hydrates - the so-called "low-salt" roots —, and in the second case the opposite: roots with a high salt content and few carbo-hydrates, the "high-salt" roots.

I could not follow this method because, as mentioned in the previous paragraph, I used an interchange of iron and phosphate, and also because I wished to compare the absorption between high-salt and low-salt plants so that the differences in growth between the two series of material should be as small as possible. For this reason I used a somewhat different method.

At first the material was treated alike, in the manner mentioned in the previous paragraph. After the plants had been put on the ferric solution, this solution was — some weeks before the experiment — replaced by tap water for the low-salt plants, and for the

high-salt ones by nutrient solution again containing phosphate (without iron).

Both solutions were renewed twice a week until the plants were used for the experiment. It is true, that treated in this way the high-salt plants are somewhat more robust than the low-salt plants, but the differences are not very marked. They may be characterised as follows:

a. Of the low-salt plants the two or three lower short leaves mostly died off; of the high-salt plants these are usually still quite green at the beginning of the experiment.

b. The leaves of the high-salt plants are somewhat robuster than those of the low-salt ones and sometimes a trifle less green. They show faint length-wise stripes on account of the zones round the nerves being dark green with somewhat lighter green stripes in between.

c. The root system of the high-salt plants mostly looks a little less white; usually the roots are also somewhat shorter and thicker.

d. If the humidity is sufficiently high, the low-salt plants display an intensive guttation during the first few days of the experiment. With the high-salt plants this exudation of water hardly ever takes place, and, if it occurs, it is far less intensive than with the low-salt plants.

As a rule the differences are so small that they only become noticeable on close comparison. However, it is not always possible to follow the method of cultivation as given above.

During the summer months in a period of abundant sunshine (such as was the case in the summer of 1947) the growth may become so rapid that in order to obtain high-salt plants the nutrient solution must be renewed every other day. The treatment of the low-salt plants also must then be a different one, since otherwise considerable differences between the two kinds of plants can not be avoided. For this reason the latter were occasionally given a diluted nutrient solution by replenishing the tapwater with phosphate containing nutrient solution. The result then is that the differences as to the salt conditions are less great, but the differences as to growth greater than normal. Thus it could happen that in some cases the accumulation at the beginning of the experiment was greater with the high-salt plants than with the low-salt ones. From the further progress of the absorption however, it appeared that this had no influence on the essential difference existing between the two kinds of material.

§ 3. *The arrangement for a continuous flow of nutrient solution.*

All the experiments were made in a room with a constant temperature of 20° C. plus or minus one degree C.

The manner in which the experiments were made differed according to circumstances. If desirable, the concentration of the nutrient solution was kept constant by resorting to the principle of continuous flow.

The method used was generally the same as that of VAN DEN HONERT (1933). Only by simplification in a few respects did it deviate from it. I will briefly explain this method (see figure 1).

The whole apparatus is mounted in a room with constant temperature, with the exception of the stock jars A which are in a similar room one storey higher. Both rooms have approximately the same temperature. For stock vessels stone jars of ten liter capacity are used, two of which are connected in series by means of a syphon. From these the nutrient solution is conducted by a syphon into a constant level reservoir B. A float keeps the level in this vessel at a constant height. From this reservoir runs another syphon which branches off into four tubes so that four series of six plants can be simultaneously kept under a constant flow. In the sketch the flow for only one series is given. In each of these branches there is a capillary C which provides the necessary resistance. By using capillaries of different length it is possible to vary the rate of flow. In order to keep a certain rate of flow as constant as possible, the capillaries are placed in a reservoir of water to eliminate small fluctuations of temperature in the room. After passing through these capillaries the solution drops from a dropper P into the culture vessels D which are placed in the same reservoir of water and have a capacity of one liter. P can be moved up and down in a stand so that if necessary a still finer adjustment of the rate may be obtained. By means of a third syphon the solution is conducted from the culture vessel into the measuring vessel. On this syphon is another dropper Q. So the outlet of this dropper is on a level with the surface of the liquid in the culture vessel. As a result of the resistance offered, the solution does not drop continuously into the measuring vessel but periodically. When the solution in the culture vessel D has slightly risen, the syphon comes into action and drips with great frequency for one or two minutes, after which it stops when the slight difference in pressure is approximately equalised. After some minutes the dripping begins anew. This period is therefore very short in comparison with the time necessary for the filling of the measuring vessel and is practically of no influence.

The measuring vessel E consists of a Wulff's bottle with three

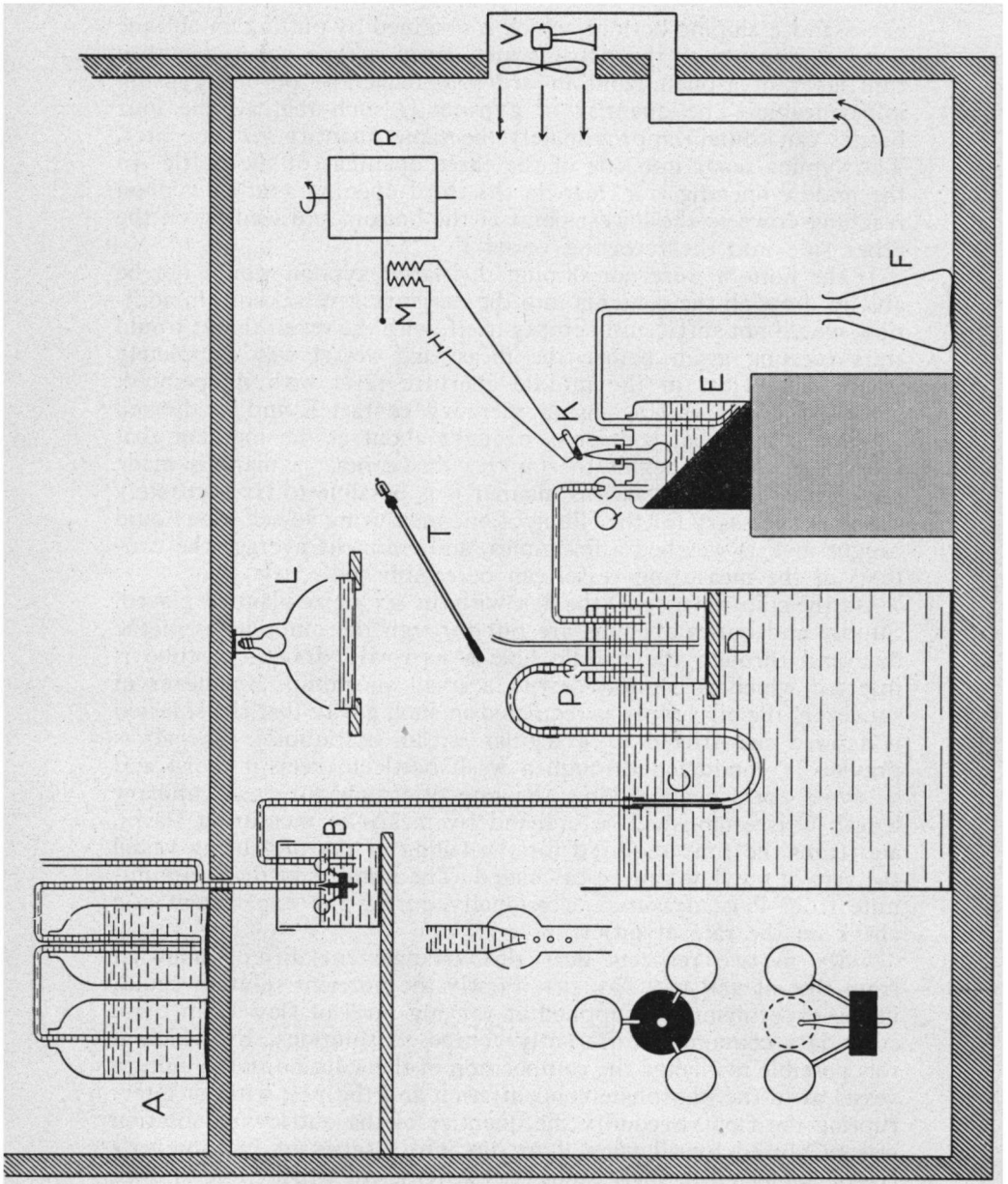

Fig. 1. Schematic representation of the arrangement for continuous flow (see text).

necks and a sloping bottom, which is obtained by putting an oblique layer of gypsum in the bottle which layer is then covered with a thin layer of asphalt paint in order to make the porous gypsum impermeable. The quantity of gypsum is such that all the four bottles can contain approximately the same quantity viz. one liter. The syphon leads into one of the three openings of the bottle. In the middle opening is a float, in the third opening another syphon reaching down to the lowest point of the bottom and leading on the other side into the receiving vessel F.

If the bottom were not sloping this latter syphon would not be able to draw all the contents into the measuring vessel and, in addition, would not sufficiently empty itself, with the result that it would start running again before the measuring vessel was completely filled. The float in the middle aperture rests with its pointed, extended upper end against a mercury contact K and is adjusted in such a way that contact is brought about at the moment that the outlet syphon begins to run. By this contact a mark is made on a kymograph, and in this manner it is possible to fix accurately the time necessary for the filling of one measuring vessel. The liquid caught in F is weighed a few times, and from the average the contents of the measuring vessel can be established.

On the culture vessel D the disc with the six maize plants is placed. Supply and outlet syphons are put through the outer holes of the disc and through the middle hole a narrowly drawn-out tube is inserted which is connected with a small air pump. By means of squeezers the air supply is regulated in such a way that the solution is aerated and stirred by a regular stream of bubbles. The air is previously conducted through a wash bottle to keep it moist and so avoid extra evaporation. The rate of inflow for each capillary length C is established beforehand by means of measuring flasks, and from the time required for the filling of the measuring vessel the rate of outflow can be calculated. The number of drips per minute from P is measured occasionally during the experiment as a check on the rate of inflow.

Only in two respects does this arrangement differ somewhat from that of VAN DEN HONERT. Firstly the nutrient solution could, in his experiment, be supplied at varying rates of flow from three stock jars containing differently composed solutions, by which it was possible to change the composition of the solution in the culture vessel as to the phosphate concentration and the pH, without interrupting the flow. Secondly the quantity of the outflowing solution was measured by allowing it to run into a series of Erlenmeyer's flasks connected in succession, the capacities of which were known.

When the first is full, the solution flows into the second etc. In this manner it was possible for him to catch the outflowing solution in separate portions also during the night, which, in my arrangement, is not the case. The simplification which I used did not, however, offer great difficulties, since in my experiment the composition of the solution was mostly kept constant, whereas the outer circumstances changed.

Light was thrown upon the plants by two electric bulbs each of 200 W. which were placed in a metal reflector at a distance of about one metre from the culture vessels. The strength of the light at the height of the plants is then about 3200 ft cdl (1 ft cdl = 10.8 lx). Under each of the electric bulbs a large Petri dish filled with water is placed to protect the plants from the heat of the lamp. On account of this rapidly evaporating water, the humidity in the room was high and the transpiration was therefore insignificant. The difference between the rates of in and outflow was usually so slight that it came within the limit of error. In this humidity, as a rule, the low-salt plants usually show an intensive guttation for the first few days. By means of the ventilator V a constant air stream was conducted past the plants. The air can then circulate in the room but air from outside may also be let in. In order to prevent the humidity from becoming excessive, the ventilator was daily connected with the outside air; in winter for only a short period (about one hour), in summer, when the temperature is sufficiently high, for the whole day. The humidity is then somewhat lower.

At a constant rate of absorption by the plants the phosphate concentration in the culture vessel will reach a constant value after the lapse of a certain time (see par. 4).

So the rate of absorption is then the difference between the product of the rate of inflow and the concentration of the inflowing solution and that of the outflow and the concentration of the solution caught: $O = v_1 . c_1 - v_2 . c_2$ *mg/h*. The water uptake per hour is then equal to $v_1 - v_2$.

If the absorption is determined from very low phosphate concentrations, these concentrations should be kept up to strength by a continuous flow, since otherwise the solution would be immediately exhausted. As, in my experiments, the phosphate concentration of the solution was usually much higher than in those of VAN DEN HONERT, I did not always use the above-mentioned method but in many experiments I determined the absorption from a stagnant solution. In this way the duration of the experiment can usually be considerably reduced, since no equilibrium need be established.

The solution was then regularly renewed in order to keep the

concentration up to strength. The absorption in this case can be calculated from the difference between the initial and the final concentration; the transpiration is not corrected because the experiments with continuous flow showed this to be very slight.

The renewal was brought about by drawing off the old solution and immediately adding one liter of fresh solution. The drawback is that the old solution can never be completely removed; a little always clings to the roots. Nevertheless the roots were not washed so that they should suffer as little injury as possible. The change in the phosphate concentration which may possibly take place in this manner will be very insignificant and has no influence whatever on the results of the experiments thus made.

§ 4. *Advantages and drawbacks of the method of continuous flow.*

The method of continuous flow was used by VAN DEN HONERT (1933) in order to determine the rate of absorption under varying circumstances. Before that time the method was applied to test the influence on the growth exercised by various nutrient solutions in different concentrations in experiments of long duration. The method has the advantage that the concentration of the solution is kept strictly constant also when a very weak concentration is used, so that it need not be renewed, which prevents injury to the roots and infection. In addition, the concentration can be modified without removing the plants or drawing off the solution.

Against these advantages there are also disadvantages which cause the principle of continuous flow to be applicable only in certain cases.

When discussing the method used by myself, I pointed out that a certain time must elapse before an equilibrium is established between the concentration of the inflowing solution, that of the outflowing solution and the absorption by the plant. VAN DEN HONERT (1930) made a calculation as to the effects of a stream of air with a certain percentage of CO_2 being conducted through a space free of CO_2. He found that a volume of gas equal to four times the volume of the given space must pass through before the CO_2 concentration of the outflowing gas is, within a 2% limit of error, equal to the CO_2 concentration of the inflowing gas. It is of no consequence whether in this space there are organisms which take up CO_2 at a certain rate. The CO_2 concentration of the outflowing gas is then somewhat lower, but the equilibrium is reached in the same time. The same applies to a solution of a definite salt concentration flowing through a vessel. So if into such a vessel containing distilled water with or without plants, some solution is led, equilibrium will not be established before four times the contents of the vessel have

passed through, i.e. that not before that time has the phosphate concentration in the culture vessel reached a constant value. It is assumed that the absorption by the plants takes place at a constant rate.

The following calculation, which is approximately analagous to that of VAN DEN HONERT, shows that the same holds good when a solution flows through a vessel originally filled with the same solution, but in which at a given moment plants are placed which take up salt at a constant rate.

Let c_i *mg/l* be the concentration of the inflowing solution; v_i *l/h* the rate of flow of the inflowing solution, and s *mg/h* the rate of absorption by the plants then $v_i t \times c_i$ *mg* matter flows into the vessel in the time t.

In the same time st mg matter is taken up by the plant.

In t seconds $v_i t \times c_i - st = (v_i c_i - s)\ t$ is added to the vessel. If we now imagine the plants to be absent, the situation remains essentially the same when a solution flows into the vessel of a concentration:

$$\frac{v_i c_i - s}{v_i} = c_i - \frac{s}{v_i} = C\ mg/l.$$

The situation is now as follows:
Vessel with concentration c_i into which flows a solution of concentration C; the outflowing concentration c_u is equal to the concentration in the vessel. When an equilibrium is established the concentration in the vessel is C *mg/l*; at the beginning $c_u = c_i$ and approaches in the course of time to C.

The question now is, what quantity of solution must have flowed through the vessel before a sufficiently accurate equilibrium is established.

If a quantity dx flows into the vessel dx also leaves the vessel. $dx \times C mg$ matter is added, $dx \times c_u\ mg$ matter is drained off. The total change is $dx\ (C - c_u)$.
The difference in concentration is:

$$\frac{dx\ (C - c_u)}{a}$$

a is the volume of the vessel

$$\frac{dx\ (C - c_u)}{a} = dc_u$$

$$dx = \frac{a \,.\, dc_u}{C - c_u} = \frac{-a \,.\, d\,(C - c_u)}{c_u - C}$$

Integration of this gives:

$$x = -a \ln (c_u - C) + k \qquad (1)$$

If $x = 0$ i.e. at the start of the experiment, then also $c_u = c_i$, therefore

$$0 = -a \ln (c_i - C) + k \qquad c_i - C = \frac{s}{v_i} = V.$$

V is thus the difference between the initial concentration and the concentration when an equilibrium is reached, thus:

$$0 = -a \ln V + k \quad \text{or} \quad k = a \ln V.$$

Substitution of k in (1) gives:

$$x = -a \ln (c_u - C) + a \ln V = a \ln \frac{V}{c_u - C}$$

$$\ln \frac{V}{c_u - C} = \frac{x}{a}$$

$$\frac{V}{c_u - C} = e^{\frac{x}{a}}$$

$$c_u = C + V \times e^{-\frac{x}{a}}.$$

In the case that $c_i = 6.5$ *mg/l*; $V = \frac{s}{v_i} = \frac{0.50 \text{ mg/h}}{\frac{1}{2} \text{ l/h}} = 3.50$ *mg/l* and $a = 1$ *l* the calculated values of c_u are given in table 1 for the successive values of x.

From this it follows that only after seven times the volume of the vessel has flowed through has the concentration of the outflowing solution become constant. After four times the volume has flowed through, the difference with the ultimate value is less than 2% (3.06—3.00).

However this is not the method I followed. The concentration in the vessel was not determined at a definite moment, but after a certain time the total amount which had flowed out in this time was analysed. This analysis takes place when a quantity has flowed out approximately equal to the contents of the vessel i.e. a quantity a.

TABLE I. Quantity of nutrient solution flowed through with corresponding calculated P_2O_5 concentration when C = 3.00 mg/l; V = 3.50 mg/l and a = 1 l (see text).

x (l)	c_u (mg/l)
0.0	6.50
0.5	5.12
1.0	4.29
1.5	3.78
2.0	3.47
2.5	3.29
3.0	3.17
3.5	3.11
4.0	3.06
4.5	3.04
5.0	3.02
5.5	3.01
6.0	3.01
6.5	3.01
7.0	3.00
7.5	3.00

The original contents of the vessel: $a \times c_i$ mg matter
added: $a \times C$ mg matter
still present: $a \times c_u$ mg matter
flowed off therefore: $a \times c_i + a \times C - a \times c_u$ (2)

$$c_u = C + V \,.\, e^{-\frac{x}{a}} = C + V \,.\, e^{-1} \quad (x = a).$$

Substitution in (2) gives

$$a\,(c_i + C - C - V \,.\, e^{-1}) = a\,(c_i - V \,.\, e^{-1}) \text{ mg matter.}$$

The concentration is thus $c_i - V \,.\, e^{-1}$ mg/l. . . . . first discharge.

The second discharge can be calculated as follows: second discharge = two discharges — first discharge.
Flowed away after two discharges:

$$a \times c_i + 2\,a \times C - a \times c_u \qquad (c_u = C + V \times e^{-2})$$

thus:

$$a\,(c_i + 2\,C - C - V \,.\, e^{-2}) = a\,(c_i + C - V \,.\, e^{-2}).$$

So the second discharge contains
$a\,(c_i + C - V.e^{-2}) - a\,(c_i - V.e^{-1}) = a\,(C + V.e^{-1} - V.e^{-2})$ mg matter.
The concentration is therefore

$C + V.e^{-1} - V.e^{-2}$ mg/l . . . . . second discharge

So the concentration of the third discharge becomes

$C + V.e^{-2} - V.e^{-3}$ mg/l etc.

TABLE 2. P_2O_5 concentration of seven successive discharges from the beginning of the experiment when C = 3.00 mg/l; V = 3.50 mg/l and a = 11 (see text).

Concentration	first	discharge	$C + V(1 - e^{-1})$	= 5.21	mg/l
„	second	„	$C + V(e^{-1} - e^{-2})$	= 3.81	„
„	third	„	$C + V(e^{-2} - e^{-3})$	= 3.32	„
„	fourth	„	$C + V(e^{-3} - e^{-4})$	= 3.11	„
„	fifth	„	$C + V(e^{-4} - e^{-5})$	= 3.04	„
„	sixth	„	$C + V(e^{-5} - e^{-6})$	= 3.02	„
„	seventh	„	$C + V(e^{-6} - e^{-7})$	= 3.00	„

Table 2 gives the calculation with the same data as for table I; the first discharge is converted as follows:

$$c_i = C + V;\ c_i - V.e^{-1} = C + V - V.e^{-1} = C + V(1 - e^{-1}).$$

From this table it is evident that the concentration of the fifth discharge deviates less than 2% from the ultimate concentration.

The same calculation naturally holds good when an equilibrium has already been established, but the plants, as a result of introducing a change, show a different, though again constant rate of absorption. Also in this case data concerning the rate of absorption by the plants can only be obtained after four times the volume of the vessel has flowed through.

From the above it is clear that the material must show a constant rate of absorption during the time necessary for an equilibrium to be established. Only in this case is, after four discharges, the difference in concentration between the inflowing and outflowing solution a reliable measure for the rate of absorption.

The time needed for the establishment of the equilibrium will depend on the rate of flow and the volume of the vessel. Both, however, are bound by certain limits. The rate of flow must be chosen in such a way that a reasonable difference in concentration remains between the in- and outflowing solution, so that the rate of absorption may be determined as accurately as possible. The volume of the vessel must always be such that it forms no hindrance to a good root development of the plant. In the experiments with sugar cane made by VAN DEN HONERT, as well as in those made by myself, it takes one day for each test.

Such a long time implies that the experiments can only be made

in a room with constant temperature, since, on account of the daily fluctuations in light, temperature and other possible factors, the rate of absorption by the material would not remain constant for twenty four hours. At all events it will be necessary to ascertain by preliminary tests that the material under the circumstances accompanying the experiment shows a constant rate of absorption. This was not always the case in VAN DEN HONERT'S experiments so that the results of several of them do not indicate the true rates of absorption but have only a relative value (see Chap. III). For the results of the majority of his experiments this is of little importance since his conclusions are based on the relative rates of absorption under varying circumstances.

In conclusion I will sum up the advantages and disadvantages of the method of continuous flow.

Advantages:

1. The concentration of the nutrient solution can be kept at a constant level, so that it is possible to determine the rate of absorption with very weak concentrations.
2. There is no risk of injuring the plant material on renewal or replenishment of the solution.
3. The data as to absorption and uptake of water can be obtained in a simple manner.

Disadvantages:

1. The rate of absorption must, under constant conditions, show no variation.
2. The experiments can only be made in surroundings where the the outward circumstances can be controlled.
3. The experiments always require a good deal of time. For instance, if, with my method of continuous flow, the rate of absorption for seven different concentrations is to be determined, a whole week will be required.
4. Short essential changes in the rate of absorption may remain unobserved with this method.

§ 5. *The phosphate analysis.*

This was carried out according to the colorimetrical method of PARKER and FUDGE (1927).

The colour intensity of the solution is measured with a photometer (Cenco-photelometer). The quantity of phosphate is determined with the aid of a calibration curve previously determined from standard solutions and expressed as P_2O_5.

By this method it is possible to demonstrate differences of 0.2 mg P_2O_5/l.

The accuracy can best be illustrated by the check tests which, in the experiments with a continuous flow, were made with regard to the phosphate concentration of the inflowing solution. Here follow the analyses of experiments 16, 17, and 20.

Test 16: four analyses; results: 6.5 — 6.5 — 6.5 and 6.5 mg P_2O_5/l.
Test 17: five analyses; results:
6.5 — 6.6 — 6.6 — 6.6 and 6.6 mg P_2O_5/l.
Test 20: four analyses; results: 6.5 — 6.5 — 6.5 and 6.5 mg P_2O_5/l.

It must here be observed that these analyses do not pertain to samples from one and the same solution, but that the figures are derived from the phosphate tests which were carried out each time a new stock of nutrient solution was made. Variations in the replenishment of the solution and possibly in the concentration of the stock solution are therefore included.

CHAPTER III.

EXPERIMENTAL.

§ 1. *General remarks on the experiments.*

All the experiments were carried out in a room with a constant temperature of 20° C.

The quantity of solution in which one set of six maize plants were placed was always one liter; the composition, unless otherwise indicated, is as follows:

KNO_3	0.0005 mol
$Ca(NO_3)_2$	0.0005 mol
$MgSO_4$	0.0002 mol
KH_2PO_4	0.0001 mol

In the case of continuous flow, the rate is approximately 130 c.c./h Further data are given for each experiment separately.

The diagrams in so far as they concern experiments in which there is continuous flow were made as follows:

From the analysis of one discharge from the measuring vessel the average rate of absorption can be calculated for the time required to fill the measuring vessel. It is now assumed that during this period the plant has taken up salt at a constant rate equal to the average

rate of absorption found. This rate is represented by a horizontal line. The distance from this line to the abscissa indicates the rate of absorption; the length of the line shows the time to which the observation refers. Occasionally (fig. 5) I did not wait for the measuring vessel to fill completely, and the line, in that case, is therefore shorter than normal; in other cases two or more discharges were combined and analysed, here the line is correspondingly longer. The course of the rate of absorption is then indicated by a curve drawn as accurately as possible through the middle of the successive horizontal lines.

§ 2. *The absorption of phosphate as to time.*

In the majority of the publications on the uptake of phosphate by intact higher plants, the growth and absorption were analysed during considerable periods and for various concentrations of the phosphate in the milieu. By this an attempt was made to give an answer to the question whether diverse economically important plants can thrive on the low phosphate concentrations found in the solution of the soil, also when these can be kept up to strength by subsequent supply: PARKER and PIERRE (1928); TEAKLE (1929); TIDMORE (1930); VAN DEN HONERT (1933); SOMMER (1936); LYNESS (1936); HOUGHLAND (1947).

Some of these writers investigated several objects simultaneously in order to ascertain whether the need of phosphate differs greatly between various species, or even between different varieties (SOMMER, LYNESS).

VAN DEN HONERT collected the results of the older experiments in a table giving the author, the nature of the object and the minimal phosphate concentration at which a satisfactory growth is still possible (l.c. pag. 1122). The lowest concentration at which such a growth is possible fluctuates between 0.05 mg P_2O_5/l (maize) and 0.5 mg P_2O_5/l (barley). Later investigators found 0.6 — 2.4 mg P_2O_5/l (SOMMER for tomatoes, wheat, buckwheat and cotton) and 1.12 mg P_2O_5/l (HOUGHLAND for potatoes).

In the majority of the experiments, however, the concentration of the outer solution was not kept sufficiently up to strength. Although the quantity of solution was large and the number of test plants small there was often a great fluctuation in the phosphate concentration; it often fell to more than half of the original strength.

This difficulty was avoided by TEAKLE and VAN DEN HONERT. Both used a solution with continuous flow. The former found for

wheat a good growth with a concentration higher than 0.75 mg P_2O_5/l and good absorption with a concentration higher than 0.37 mg P_2O_5/l. It was a remarkable fact that the uptake practically no longer increased with a concentration higher than 0.37 mg/l, but that a good growth only set in with a concentration above 0.75 mg/l.

VAN DEN HONERT used sugar cane. Compared with other investigators he made experiments of short duration whilst he tested only the uptake of phosphate and not the growth. The different concentrations were successively administered to the same object and the hourly rate of absorption calculated. Each determination took a day so that one experiment took about a week. In this way it is possible to compare more accurately the rates of absorption with different concentrations.

These experiments being little known in the English literature on the subject, since they were published in Dutch, I will here briefly deal with the principal results that are of importance in this connection. With the above mentioned method of continuous flow, the concentration of the nutrient solution could be kept exactly at a certain strength and also be varied without injury to the plants. This is also the case with pH. From the experiments it appears that the connection between the rate of absorption and the concentration is represented by a saturation curve: with a concentration of 1.0 mg P_2O_5/l the rate of absorption is already at its maximum, and with higher concentrations it keeps the same value. As the curve found shows a great resemblance to Freundlich's adsorption-isotherm, VAN DEN HONERT assumes that the intensity of the uptake depends on an adsorption process. He conceives that the process of absorption begins with an adsorption of ions from the solution on the plasm-surface of the root. He has made it clear that with a normal adsorption process an equilibrium is established between adsorbed and free ions, but that in an absorption process we have to do with the connection between the concentration of the ions in the medium and the rate of absorption, i.e. the quantity of ions which are taken up in a given time. This leads him to the conception that the ions after being adsorbed on the root surface are, at a constant rate, carried inward from this surface so that a new equilibrium between free and adsorbed ions can constantly be established on the surface layer. The rate of absorption by a root system would then depend on the adsorption equilibrium and the rate at which the ions are carried away from the surface.

The first factor is dependent on the concentration of the solution, the second on the temperature and, in addition, (though this is not

mentioned by VAN DEN HONERT) on the supply of carbohydrates and oxygen. (c.f. HOAGLAND and BROYER 1936).

In the experiments made by VAN DEN HONERT, pH proved to be of influence also on the absorption. This pH influence could be explained by assuming that only the H_2PO_4 ions were absorbed. It now became evident that, independent of pH and the temperature, the H_2PO_4 ion concentration, above which no further increase of the rate of absorption takes place and whereby the adsorption on the surface layer is therefore at its maximum, had invariably the same value and consequently for sugar-cane represents a constant. VAN DEN HONERT took as a characteristic point the so-called half value, i.e. the concentration whereby the absorption under given conditions is half of what is maximal under identical conditions. This value is for sugar-cane 7.5×10^{-6} g ion H_2PO_4. He presumes that every plant, as far as the absorption is concerned, may be characterized by such a value.

As, with a concentration of 1 mg P_2O_5/l the phosphate absorption has already reached a maximum rate, VAN DEN HONERT infers that in the case of sugar-cane good growth will still be possible with a considerably lower concentration, and that it is not necessary to assume, as was done by PARKER and PIERRE (1928), that the concentration in the soil solution is insufficient for good growth. This conclusion, however, regards only the Saccharum variety examined by VAN DEN HONERT.

So from all these investigations it appears that with all kinds of plants the growth is bound up with a given minimum concentration. This concentration may be different for different plants, and according to an investigation made by LYNESS (1936) may differ considerably even in various maize varieties.

Only few papers give data concerning the progress of absorption as to time.

RADU (1936, 1937) finds that with alfalfa and maize during the whole period of vegetative growth phosphate is taken up, though the absorption is not always equally great. With maize, according to him, it first rises to a given maximum after which it gradually falls during the further development of the plant.

HOUGHLAND (1947) finds that during the whole period of growth for potatoes phosphate is needed, that there is no correlation here between the magnitude of the absorption and the periodicity of the plant. Thus, for instance, no change as to the absorption of phosphate could be found during the time of strong growth of the young tubers. According to him there seems rather to be a connection between the absorption and the external circumstances. VAN DEN HONERT

finds the same (1933). The absorption of phosphate by sugar-cane goes on for some days at approximately the same average rate, but, as a result of the varying external circumstances, there are considerable differences between day and night.

When discussing the method, I have already mentioned that, when changes in the rate of absorption occur, the values found with the method of continuous flow present a delayed picture of these changes (c.f. also par. 4), so that the rate of absorption calculated for a given period does not give the true rate of absorption during that time, since this was not constant for the time necessary for the establishment of an equilibrium (about twenty-four hours).

This difficulty, however, will not have had much influence on the conclusions drawn by VAN DEN HONERT, since the samples that were compared with each other were always taken at the same time of the day. But the conclusion come to in 1933 that the rate of absorption is independent of the transpiration is not justified on these grounds. However, the experiments were later (1936) repeated by him under constant conditions whereby the daily fluctuations were eliminated; they had the same results.

In order to ascertain whether the absorption of phosphate by maize plants can, under constant conditions, proceed for some days at the same rate, the following experiment was made.

On 1/7/'47 an experiment was commenced with four sets of six maize plants, forty six days old, two sets of which were in high-salt and the two others in low-salt condition. There was a continuous flow and aeration of the nutrient solution; there was also continuous exposure to light. When possible each discharge was analysed; where this was not possible (at night) some discharges were combined. The results are shown in fig. 2. The separate observations for high-salt and low-salt plants are averages of two sets. From the data it is evident that there is a great difference in the rate of absorption between the two kinds of plants. With the low-salts plants it is about double that of the high-salt ones. It further appears that after an initial increase the rate of absorption in both kinds of plants remains approximately constant during the further progress of the experiment. With the high-salt plants the initial increase is not a true one but a result of the method of continuous flow (cf. Chap. II par. 4). After the fourth discharge the curve already runs parallel to the abscissa. With the low-salt plants, on the other hand, the increase is a true one, for it continues until the eighth discharge and only then does the absorption become constant.

This experiment was repeated several times and invariably with the same result; only the quantitative differences between the two

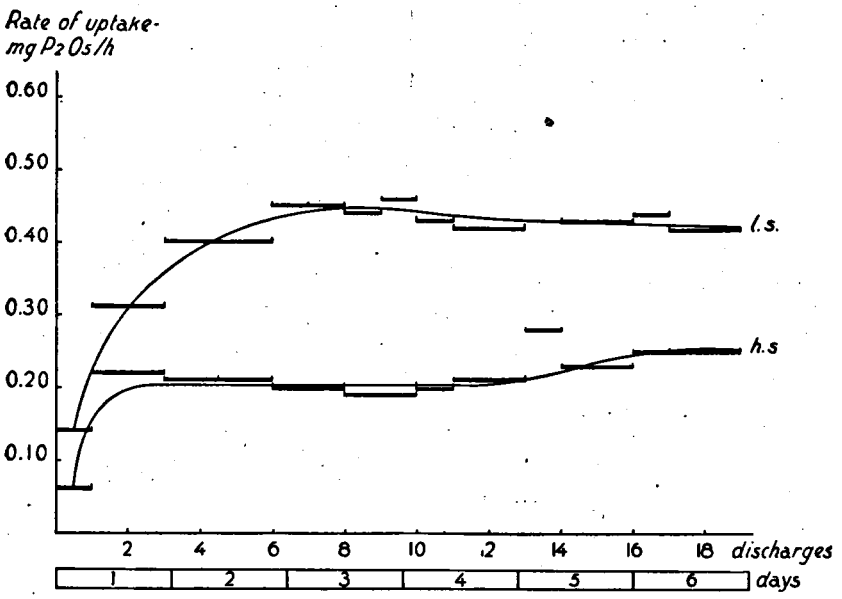

Fig. 2. The course in time of the rate of phosphate absorption by two sets of high-salt and two sets of low-salt plants.
Continuous flow.
Separate values are averages of two sets.
h.s. = high-salt; l.s. = low-salt.

kinds of material may vary. The shape of the curves therefore remains the same, but the distance between them may vary. This becomes evident also from the results of the following experiment commenced on the 27/8/'47 with two sets of six low-salt maize plants and two sets of six high-salt ones, all thirty three days old. In order to avoid the initial increase with the high-salt plants, as a result of the method of continuous flow, this method was not used in this case, but the solution was drained off every twelve hours and replaced by a fresh solution of the same composition. There was continuous aeration and exposure to light. The absorption was determined from the difference between the initial and final concentrations, and the values found are indicated by dots half way in the twelve hours period (fig. 3) as averages of two sets of high-salt and low-salt plants.

Although in this case, as a result of climatic conditions during the cultivation, the rate of absorption of the low-salt sets is at first lower than that of the high-salt ones (cf Ch. II par. 2), yet the pro-

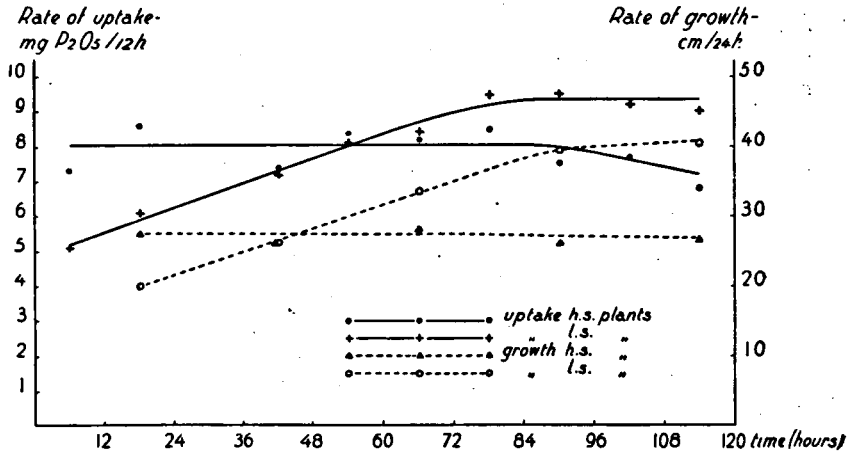

Fig. 3. The course in time of the rates of phosphate absorption and growth by two sets of high-salt and two sets of low-salt plants.
No continuous flow; solution renewed every twelve hours.
Separate values are averages of two sets and indicated halfway the twelve hours' period.

gress of the rate of absorption as to time is again the same as in fig. 2. With the high-salt plants the rate of absorption remains unaltered during the experiment; with the low-salt plants there is an increase during the first three days and only then does the rate of absorption become constant.

The curves of growth in this diagram will be discussed in par. 5.

§ 3. *The connection between the absorption and the concentration of phosphate.*

The number of investigations into the connection between absorption and concentration is considerable. Without pretence to being exhaustive I will mention POUGET and CHOUCHAK (1912), STILES and KIDD (1919), HOAGLAND, DAVIS and HIBBARD (1928), GRACANIN (1932), STEWARD (1933), VAN DEN HONERT (1933, 1936, 1937), COLLANDER (1936), ARISZ (1944, 1947), SCHUFFELEN and LOOSJES (1947). From all their researches it appears that the absorption with low concentrations is comparatively much greater than with higher concentrations.

On account of the fact that the connection between absorption and concentration greatly resembles Freundlich's adsorption isotherm, many of the above-mentioned investigators have arrived at the conclusion that with the absorption an adsorption process is linked up. POUGET and CHOUCHAK are exceptions; they surmise that

in a concentrated solution, the absorption depends on the consumption of the salts in the plant but that in more diluted solutions the concentration regulates the absorption, so that with increasing concentration the absorption increases until other factors in the plant become limiting and the concentration has no longer any influence.

In fig. 4 the result is shown of an experiment made on 6/11/'47 with two sets of high-salt and two sets of low-salt plants. Here the phosphate concentration varied between 1.3, and 35.8 mg P_2O_5/l; the concentration of the remaining salts was always normal. The solutions were given in an increasing concentration of phosphate, each for two hours. Every two hours the solution was drained off and replaced by a new solution with a higher P_2O_5 concentration. The successive concentrations were 1.3 ; 6.3 ; 12.0 ; 25.0 ; and 35.8 mg P_2O_5/l.

Exposure to light and aeration of the solution are continuous. From the difference between the initial concentration of the solution and that after two hours the rate of absorption is determined and, for each set separately, marked against the average between the initial and the final concentrations. This method has the disadvantage that the concentration cannot be kept sufficiently up to strength,

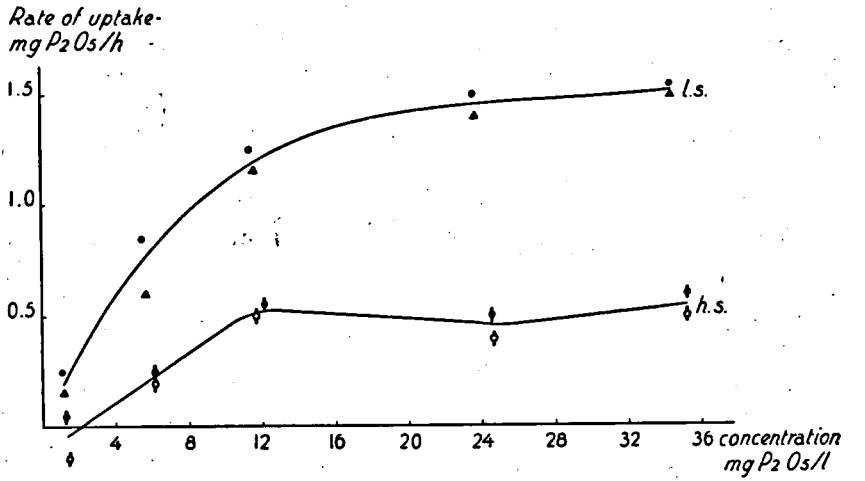

Fig. 4. The relation between the rate of absorption and phosphate concentration in the culture solution with two sets of high-salt and two sets of low-salt plants.
No continuous flow; each concentration administered for two hours.
Values are indicated for each set separately.
h.s. = high-salt; l.s. = low-salt.

but it has the advantage that the time taken by the experiment is very short, so that there is no risk of the low-salt plants changing into high-salt ones during the experiment.

From the curves in fig. 4 it can be deduced that with all concentrations the absorption by the low-salt plants is much higher than that by the high-salt plants (about three times as high), but also, that the absorption by the high-salt plants is already independent of the concentration when this is higher than 12.0 mg P_2O_5/l, whereas with low-salt plants this is not the case until a concentration of about 24.0 mg P_2O_5/l is attained.

At first I assumed the concentration above which the absorption no longer increases to have about the same value as was found for *Saccharum* by VAN DEN HONERT and therefore the phosphate concentration was at first brought up to only 6.5 or 13.0 mg P_2O_5/l. Thereby, however, a maximum absorption was never found for the low-salt plants, whereas with the high-salt ones it was found more often than not. From this it becomes evident that the concentration above which there is no further increase in absorption depends on the nutritional state of the plants and is presumably determined by internal factors, such as the extent of the previous salt supply and the carbo-hydrate metabolism.

§ 4. *The phosphate absorption in light and in darkness.*

Various investigators have inquired into this subject: HOAGLAND and DAVIS (1923); HOAGLAND, HIBBARD and DAVIS (1923); NEMEC and GRACANIN (1926); ROSENFELS (1935); INGOLD (1936); SCHMIDT (1936/7); COLLANDER (1939); LUTTKUS and BÖTTICHER (1939); BÖTTICHER and BEHLING (1940); ARISZ (1947).

They all found that light has a stimulating influence on the absorption of salt. Two of these writers have analysed the influence of light on the absorption of phosphate by intact higher plants viz. SCHMIDT for Coleus and LUTTKUS and BÖTTICHER for maize. SCHMIDT examined the absorption of various ions by Coleus cuttings under different degrees of light, but with constant transpiration. He found that in strong light there is good absorption of phosphate but that by declining light there is exosmosis of phosphate to the external solution; in darkness he found a slight absorption. Not all the ions examined behaved in the same manner; the connection between absorption and intensity of light is represented for Ca, PO_4 and NO_3 by a minimum curve; for K and Mg, on the other hand, a saturation curve is found.

For maize LUTTKUS and BÖTTICHER have compared the absorption of various ions in light and in darkness. Thereby it appeared that

there is a good absorption in light but that in darkness there is often, but not invariably, a loss of phosphate through the roots to the solution. Here, too, it was evident that the influence of light was different for different ions. Of the ions examined, the influence of light was strongest for K, whilst in darkness there is a considerable exosmosis of this ion. On the other hand, the influence of light is very small for Ca.

The other investigators dealt with other ions, and often did not work with entire plants (HOAGLAND and coworkers with *Nitella* cells; ROSENFELS and INGOLD with terminal shoots of *Elodea*; COLLANDER with *Chara* cells; ARISZ with *Vallisneria* leaves).

In all cases where potassium ions were examined a very strong influence of light could be observed.

The question may arise whether the exosmosis or the stagnation of the absorption in darkness is not the result of some injury to the material. LUTTKUS and BÖTTICHER, however, found that such was not the case, since in a second period of light following a period of darkness the absorption returned to the original level.

In a provisional experiment I examined whether with maize there is in darkness a decrease in the rate of absorption and, if so, whether this absorption may be restored during a following period of light. For this purpose an experiment was begun on 27/10/'43 with one set of six maize plants twenty nine days old. There was continuous flow and aeration of the solution. At first the plants were continuously exposed to light. The analysis was begun on 28/10. The successive discharges, or sometimes part of a discharge, or some combined discharges were analysed. Forty four hours after the commencement of the expriment there followed a ninety seven hours' period of darkness, after which the plants were again exposed to light until the end of the experiment.

We see from fig. 5 that, in darkness, the rate of absorption gradually decreases and finally comes to a standstill. Then, under the exposure to light, the absorption is resumed and at the end of the experiment it has returned to its original rate. The experiment was twice repeated with identical results. It must here be pointed out that, as a result of continuous flow, the standstill in absorption is reached sooner than is shown in the diagram. Also the original level of the rate of absorption is, under exposure to light, reached earlier than appears from the diagram. From the fact that, after a dark period of some days, the absorption returns to its original level under exposure to light, it is evident that also in this case the stagnation in the absorption of phosphate during darkness is not the result of injury to the plants. However, it did appear from later

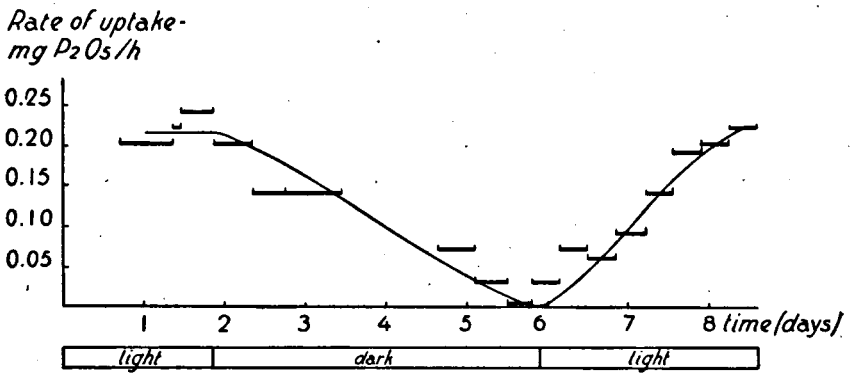

Fig. 5. The rate of phosphate absorption by one set of high-salt plants in successive periods of light and darkness.
Continuous flow.

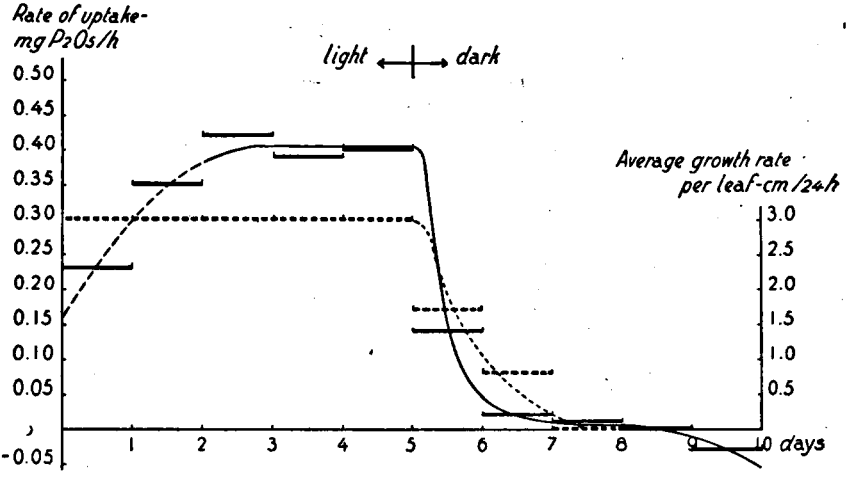

Fig. 6. The course in time of the phosphate absorption and growth in light and in darkness by four sets of high-salt plants.
Continuous flow.
Separate values are averages of four sets.
Full drawn line = rate of uptake (broken part not significant) Dotted line = rate of growth.

experiments that the period of darkness must not be continued for more than about five days; after a week the plants become limp and begin to wilt.

The same becomes clear from the two following experiments, whereby, once again, the rate of absorption in light and in darkness was examined. The first experiment was commenced on 15/5/'46 with four sets of high-salt maize plants fifty two days old. There was continuous flow and aeration of the solution.

Immediately after the commencement of the experiment the analysis was begun. The discharges of one day were caught, combined and analysed. The results of the four sets are averages (fig. 6). The second experiment was begun 5/9/'46 with four sets of six high-salt maize plants twenty seven days old. There was continuous aeration of the solution and, at first, also a continuous flow. From the 9/9/'46 onwards, each day a sample of 10c.c. was taken from the culture vessels, of which the P_2O_5 percentage was determined. On the 13 September the light was switched off and, simultaneously, the flow was stopped, in order better to demonstrate a possible

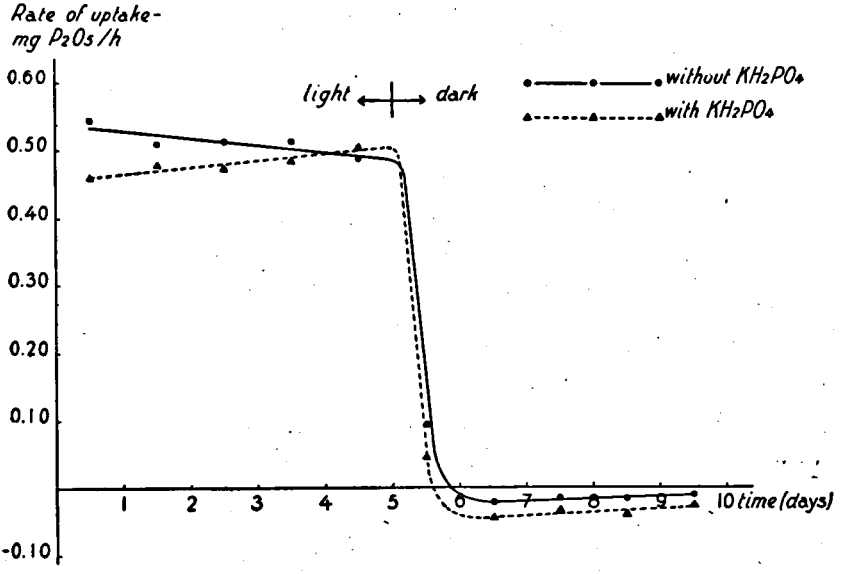

Fig. 7. The course in time of the rate of phosphate absorption in light and in darkness by four sets of high-salt plants.
In light continuous flow; in darkness flow was stopped.
To two sets a 1% KH_2PO_4 solution was administered through the leaves.
Separate values are averages of two sets.

exosmosis. The analyses proceed further in the same manner (fig. 7). Both experiments will be further discussed in the next paragraph.

From the curves representing the rate of absorption in light and in darkness, it appears that in light good absorption is possible, which, during this period, takes place at an approximately constant rate. The initial increase in the rate of absorption in fig. 6 is for the greater part a result of the method of continuous flow. In darkness the rate of absorption falls very rapidly and then passes into a slight loss of phosphate to the external solution. In fig. 6 the decrease in absorption as the result of a continuous flow, is more rapid than is evident from the diagram, and, moreover, the exosmosis takes place earlier; from fig. 7 it appears that absorption may pass into exosmosis within twenty four hours.

From these experiments we may conclude that light has a great influence on the absorption of phosphate by high-salt plants. In darkness the plant is not capable of taking up phosphate; in most cases a small quantity then passes into the solution. From the fact that this loss sets in within twenty four hours after the commencement of the dark period, and from the data in figure 5, from which it is manifest that the darkness may continue for some days without injuring the plant, we may conclude that the loss is not a result of damage to the material.

§ 5. *The connection between absorption of phosphate and growth.*

HOAGLAND and BROYER (1936) pointed out, in connection with their own experiments and those of Steward and coworkers, that fullgrown cells can take up a limited quantity of ions and that, therefore, the absorption by a plant or tissue decreases after a lapse of time, unless growth takes place.

OVERSTREET and JACOBSON (1946) are, on this point, more or less of the same opinion: "An important aspect of the ion absorption process, as revealed by past experiments, is its dependence on growth and on the active metabolism of the plant". (p. 107).

If this be correct, the absorption by intact plants can only continue for a lengthy period when there is growth, unless in some way or other (e.g. secretion through the leaves) the plant can get rid of the absorbed ions. If we now find differences in the rate of absorption between low-salt and high-salt plants these may, according to the above-mentioned conceptions, be the result either of a difference in growth or of a difference in the root metabolism. For this reason some experiments were made whereby the difference in rate of growth and the difference in the percentage of carbo-hydrates between low-salt and high-salt plants were determined.

In my experiments the rate of growth could scarcely be determined from the increase in weight during a given period since injury to the material might have resulted. So I had to look round for another method. However, the growth of grasses, and also of maize, is a very complicated process, as has been demonstrated by KUYPER (1915) and others. There is growth of the stem as well as of the young leaf sheaths and leaf blades of the young plant. Of the stem the youngest still growing part is concealed in a tube formed by the leaf sheaths and very young leaves, and its growth can therefore not be measured. The same difficulty applies to the leaves; the oldest leaves are completely visible but no longer grow; according as the leaves are younger the leaf sheath becomes gradually invisible because it is partly enclosed in that of the older leaves, and finally also the leaves partly disappear into the tube formed by the leaf sheaths, so that only the extreme tips of the youngest leaves can be seen.

According to KUYPER the order is as follows: first the lamina develops, then full development of the sheath takes place and finally the internode belonging to the leaf begins to stretch. The growing stem parts and leaf sheaths are therefore completely invisible and, of the youngest leaves, only the top is to be seen.

It is not possible, as in the case of Dicotyls, to determine the increase in length during successive periods from the distance between two given points. It would, for instance, not be correct to measure the change in length between the base of the stem and the top of a given young leaf, since, also by constant growth of the plant as a whole, the increase in length would gradually diminish until it came to a stop when the leaf was full grown.

For this reason first the visible part of one of the youngest leaves was measured i.e. from the top to the point where it disappears into the still partly rolled-up preceding leaf. When this length had exceeded 20 c.m. the next youngest leaf was passed on to, so that the measurements were always taken on leaves of approximately the same age. The data thus obtained were rather irregular; if, however, an average was taken of all the plants used in the experiment, this proved to remain more or less the same under constant conditions.

By this method the growth was determined in experiment 6.

As the increase in length of such a leaf fragment is not only determined by the growth of the leaf measured, but also by the gradual unrolling of the preceding leaf, a better method of determining the rate of growth was looked for.

Now, according to KUYPER, the place of the youngest visible leaf

joint i.e. the transition between blade and sheath, is a sufficiently fixed point. The increase in length was now determined by the distance between this point and the top of the youngest visible leaf. For this purpose these two points were marked at the beginning of the experiment and on successive days the distance between them was regularly measured. It was found that the distance between the wooden cover of the culture vessel and the marked joint practically no longer changed. The only advantage of the latter, as a fixed point, over the former is that the distance to the youngest leaf is shorter and therefore more easily measured. In the course of the experiment one or more leaf joints became visible, but the marked joint was kept as a fixed point. On the other hand, the next leaf was passed on to when the distance measured exceeded 40 c.m. The figures for growth obtained in this way were noticeably more regular.

The progress of the rate of growth was determined in the experiments 3 and 6 which have already been partly discussed.

In figure 3 the lines drawn represent the course of the rate of absorption as to time, for high-salt and low-salt plants.

The broken lines give the course of the rate of growth. This is found by determining for each plant the increase in length of the youngest visible leaf for twenty four hours, and adding up these values for one set of plants. The dots are again placed in the middle of the twenty four hours' period and are the averages of the results for two sets of plants. We notice from the diagram that the curves for absorption and rate of growth run practically parallel to each other both with low-salt and high-salt plants.

In figure 6 the rate of absorption in light and in darkness is given (the full drawn line). The rate of growth is here determined by measuring the increase in length during twenty four hours from the top of the youngest visible leaf to the point where the leaf emerges from the rolled up part of the preceding leaf, adding up these values for the four sets of plants and dividing the sum total by the number of leaves measured. Here, the rate of growth per leaf in twenty four hours is given. In this case, too, the curve representing the rate of growth (broken line) practically runs parallel to that of the rate of absorption. In darkness there is, therefore, no absorption and no growth of the shoots.

As to the differences in the percentage of carbo-hydrates in the roots, we have already data of HOAGLAND and BROYER (1936) who found that their low-salt barley roots had a much higher percentage of carbo-hydrates than the high-salt ones.

Later (1940) HOAGLAND explains this by assuming that, by the presence of sugars, the osmotic value of the cell sap is kept up to strength.

LYNESS (1936) and BEHRENS (1939) find that plants with a deficiency of phosphate have a higher percentage of sugar than normal, especially of sucrose. BEHRENS explains this by assuming that with deficiency of phosphate the breaking down of sugar is hindered, which causes accumulation.

I examined the percentage of sugar in my maize roots by approximately the same method as HOAGLAND and BROYER. On 24/11/'47 the roots of two sets of high-salt and of two sets of low-salt plants, sixty nine days old, were cut off immediately below the seed, washed in distilled water, and freed from adherent water by keeping them wrapped in a sheet of filter paper for one minute. They were then immediately weighed and frozen at a temperature of —15° C. In two days they were one by one thawed and squeezed out in a micro-press up to 400 atm. The almost clear expressed sap was caught in a measuring flask of 50 c.c. and made up to volume with distilled water. The percentage of total sugar in this expressed sap was determined by means of the method of HAGEDORN and JENSEN. The results are given in table 3.

TABLE 3. Total-sugar content of two sets of high-salt and two sets of low-salt plants.

Set	Fresh weight in g.	Total-sugar in mg.	Total-sugar in % fresh wt.
h.s. 1	18.9	297.5	1.57
h.s. 2	17.1	215.0	1.26
l.s. 1	15.1	430.0	2.85
l.s. 2	15.0	465.0	3.10

The total-sugar contents are given here in a percentage of fresh weight. From this it appears to be in agreement with the data of HOAGLAND and BROYER that the roots of low-salt plants contain twice as much sugar as those of the high-salt plants.

From these experiments we see that in all the cases examined there is a close connection between the rate of absorption and the rate of growth, and also, that with low-salt plants the percentage of carbo-hydrates in the roots is greater and their growth stronger than is the case with high-salt plants. Moreover, it appears that with high-salt plants, the growth and absorption soon come to a standstill in darkness. The following experiment was made to examine whether, in the case of low-salt plants, which have a so much higher percentage of carbo-hydrates in their roots than the high-salt plants, growth and absorption are at all possible in darkness.

On the 30/7/'46 an experiment was begun, with four sets of six

low-salt plants, thirty two days old. There was no continuous flow and no aeration; from the beginning of the experiment the plants were in darkness. Twenty four and forty eight hours after the commencement of the experiment, a sample of 10 c.c. was analysed for the percentage of phosphate; after the lapse of forty eight hours the solution was renewed and again a sample was drawn after twenty four and forty eight hours. The results are given in table 4.

TABLE 4. Phosphate uptake in darkness by four sets of low-salt plants. No continuous flow.

Time	Set I		Set II		Set III		Set IV	
	a	b	a	b	a	b	a	b
At the start	6.0	—	6.0	—	6.0	—	6.0	—
After 24 hours	2.4	3.6	5.0	1.0	2.7	3.3	3.8	2.2
After 48 hours	0.0	2.4	2.6	2.4	0.0	2.7	0.0	3.8
After renewal	6.0	—	6.0	—	6.0	—	6.0	—
After 72 hours	3.2	2.8	2.2	3.8	0.0	6.0	2.4	3.6
After 96 hours	1.0	2.2	0.0	2.2	0.0	—	1.4	1.0

a = concentration of the solution in mg/l
b = rate of uptake in mg/24h

From this it became evident that in darkness low-salt plants are indeed capable of taking up phosphate; forty eight hours after the beginning of the experiment the phosphate concentration in the nutrient solution had fallen to zero in three of the four sets of plants. After the lapse of another forty eight hours this was again the case in two of the four sets. Here the growth has not been given in figures. It could be ascertained, however, that in darkness there had been sufficient growth. During the experiment some leaves of each plant had unfolded and these were greatly etiolated; the older leaves showed a distinctly light green leaf base. It is possible that the carbohydrate content of the root, which is greater here than in the case of high-salt plants, has an influence on the rate of uptake and, indirectly, on the rate of growth. For when the percentage of carbohydrates is large enough, growth and absorption are possible also in darkness.

In order to obtain confirmatory proof for these opinions I tried to cause the growth to be resumed in darkness by administering carbo-hydrates to high-salt plants, and to discover its influence on the absorption. I did not think it advisable to add sugar to the nutrient solution in view of the development of bacteria. However, WENT and BONNER (1943) report, as the result of their experiments, that it is possible to administer sugar to a plant through the leaves

and to strengthen the growth in this manner. In the same way SPOEHR (1942) succeeded in further cultivating albino maize and even in making it flower.

By means of SPOEHR'S technique I tried to influence the growth in darkness. For this purpose the extreme tops of the leaves were cut off and the cut extremities plunged into small sterile vessels with a sterile sucrose solution, 0.3 mol. The vessels were daily replaced by others (also sterile) and again filled with the sugar solution. The zone of the leaf (about I c.m.) which had been in contact with the solution was cut away so that the leaves came each day into the solution with a new surface and a new cut.

Although at the end of the experiment the plants treated with the sugar solution had a somewhat better appearance than the sets without sugar, a difference in rate of growth either in light or in darkness could not be found; nor was there any difference in the absorption of phosphate. In both cases a slight loss of phosphate in darkness remained noticeable.

The loss of phosphate in darkness, which I always found, was also observed in comparable experiments by SCHMIDT and LUTTKUS and BÖTTICHER. The loss of ions by plants is a frequent occurrence, especially after flowering has begun. For literature in detail on this subject see LUTTKUS and BÖTTICHER.

I have attempted to analyse this process of exosmosis more precisely. The last mentioned investigators assume that exosmosis takes place as a result of a concentration gradient. They find that a maize plant one half of whose root system is placed in a very weak solution and the other half in a more concentrated one, loses in darkness more potassium to the former solution.

Starting from this assumption, I tried to increase the exosmosis in darkness by letting the leaves absorb phosphate from a KH_2PO_4, solution, in order to obtain a stronger concentration gradient and ascertain whether this was of any influence on the exosmosis by the root.

To this end an experiment was commenced on the 5/9/'46 with four sets of six high-salt maize plants twenty seven days old, which I have already discussed in paragraph four. There was continuous aeration and, at first, also continuous flow. In the beginning the plants were exposed to light, and from the 9/9/'46 a sample of 10 c.c. was taken daily from the culture vessels, and the P_2O_5 percentage determined. On 11/9 a 1% KH_2PO_4 solution was given to two of the four sets in the following manner:

Of each of the six plants of one set, one leaf was plunged into a vessel with KH_2PO_4 solution, which was placed between the plants by

the aid of a stand. By means of plugs of cotton wool the leaves were loosely inserted into the opening of the vessel. The volume of the solution was about 50 c.c. and the leaves were over a length of 8 to 10 c.m. in contact with the solution. Every forty eight hours the phosphate solution was renewed. On the 13/9 the light was switched off and the flow was simultaneously stopped so that possible exosmosis could be more easily shown.

Further, the analyses proceeded in the same way. The results are given in fig. 7. The dots are the averages of the analyses of two sets with their leaves in the KH_2PO_4 solution, and of two sets where such was not the case.

The process of absorption in light and darkness was the same in both cases, and identical with that of former experiments. It is true that the exosmosis to the nutrient solution is greater with plants to whose leaves phosphate is supplied than with plants where this is not the case, but this difference is not significant and may be a result of the variability of the material (cf. the difference in rate of absorption in light).

In addition, the leaves proved to have absorbed phosphate from the solution. Nor did the absence of phosphate in the nutrient solution in darkness, so as to obtain a greater concentration gradient, affect in any way the exosmosis. A further analysis of the factors governing this exosmosis process was not made.

§ 6. *The connection between the absorption of phosphate and the concentration of the accompanying ions.*

In the experiments made by VAN DEN HONERT and myself concerning the connection between absorption rate and concentration, only the KH_2PO_4 concentration in the solution was changed, with the result that with the stronger phosphate concentration, the proportion between phosphate ions and other ions was modified. Now it is a well-known fact that this proportion in a normal physicochemical adsorption process of inorganic ions on a given adsorbent, is of real influence, since here an ion is capable of driving another similarly charged ion away from the surface layer.

The question may be asked whether in my experiments on the connection between absorption and concentration, expulsion phenomena were not studied at the same time.

Therefore, a similar experiment as that in Par. 4 was made later on, in which, however, the concentration of the other ions was also modified to such an extent that the proportion in which the various ions occur in this solution, remained the same. This, however, had

no influence on the shape of the curves, which fully corresponded with those of figure 3.

Subsequently, this was more closely examined by giving the plants two solutions in succession, in which the phosphate concentration was the same, but the concentration of the accompanying ions differed. The absorption of phosphate was determined in both cases and the results compared.

TABLE 5. The influence of the concentration of the accompanying ions on phosphate absorption by two sets of high-salt and two sets of low-salt plants.
No continuous flow; solution renewed every two hours.

Number	Initial concentration mg P_2O_5/l	l.s. Set I Uptake mg P_2O_5/2h	h.s. Set II Uptake mg P_2O_5/2h	l.s. Set III Uptake mg P_2O_5/2h	h.s. Set IV Uptake mg P_2O_5/2h
Ia	12.7	1.8	1.5	1.4	1.6
Ib	12.8	1.8	1.5	1.4	1.6
IIa	6.7	1.7	0.5	1.3	0.6
IIb	6.6	1.7	0.5	1.1	0.5
IIIa	6.5	1.5	0.4	0.8	0.5
IIIb	6.5	1.5	0.4	0.5	0.5

Composition of the solutions:

Ia	KH_2PO_4	0.00020 mol	Ib	KH_2PO_4	0.00020 mol
	KNO_3	0.00050 „		KNO_3	0.00013 „
	$Ca(NO_3)2$	0.00050 „		$Ca(NO_3)_2$	0.00013 „
	$MgSO_4$	0.00020 „		$MgSO_4$	0.00005 „
IIa	K_2PO_4	0.00010 mol	IIb	KH_2PO_4	0.00010 mol
	KNO_3	0.00025 „		KNO_3	0.00130 „
	$Ca(NO_3)2$	0.00025 „		$Ca(NO_3)_2$	0.00130 „
	$MgSO_4$	0.00010 „		$MgSO_4$	0.00050 „
IIIa	KH_2PO_4	0.00010 mol	IIIb	KH_2PO_4	0.00010 mol
	KCl	0.00025 „		KCl	0.00130 „

The numbers I, II and III refer to three experiments with different sets of plants (see text).

In table 5 the results of three experiments, all made in the same manner but in each case with different material, are combined. The plants were first placed on solution a; in two hours this was drained off and replaced by solution b which, in its turn, was drained off in two hours. Only the concentrations of the accompanying ions differed in the solutions a and b. In this way the influence of the

concentration of the accompanying ions on the phosphate concentration could be verified.

Experiment one was made on 28/9/'47 with two sets of six high-salt maize plants and two sets of six low-salt ones, thirty one days old. Experiment two was made on 10/10/'47 with two sets of six high-salt maize plants and two sets of six low-salt ones, forty one days old.

Experiment three was made on 23/10/'47 with two sets of six high-salt maize plants and two sets of six low-salt ones, forty six days old.

In experiment I, the proportion of the accompanying ions is $a : b = 4 : 1$.

In experiment two: $a : b = 1 : 5$.

In experiment three also $a : b = 1 : 5$.

In the table the results of the experiments and the composition of the solutions are given. This also shows that the absorption of phosphate, by high-salt as well as low-salt plants, is entirely independent of the concentration of the other ions: viz. NO_3, SO_4, and Cl. This points to the fact that when the beginning of the absorption process is an adsorption on the plasm surface, this adsorption is nevertheless not identical with the adsorption process on a non-living adsorbent.

These phenomena are not new. HOAGLAND, DAVIS and HIBBARD (1928) had already found that the accumulation of Br ions by *Nitella* cells is not influenced by the concentration of SO_4, PO_4, or NO_3 ions but that such is indeed the case by the presence of Cl or J ions.

As phosphate and nitrate are scarcely absorbed by Nitella cells whereas these cells do absorb halogen ions, the writers come to the conclusion that the absorption of a given ion may be hindered by the simultaneous absorption of other ions. An ion that is capable of being easily accumulated by a plant may therefore be able to influence the absorption of another ion. An ion that is not easily absorbed cannot do so. This competition between the ions was more closely investigated for Br and J. It appeared that from a solution with a given concentration of Br ions Br is better absorbed than Br plus J from a solution containing the same concentration Br plus J. From this it seems to be evident that the ions have a hampering effect on each other.

As a second inquiry into this subject, COLLANDER'S work (1941) must be mentioned. This, too, makes it clear that the absorption of a given ion proceeds independently of the concentration of other similarly charged ions in the solution. In particular, the absorption of various cations was examined by Avena, Helianthus and Pisum.

Here too, there are certain ions to which this independence does not apply. Thus it was found that the absorption of potassium is influenced by the presence of Rb and Cs in the solution, and the Ca absorption by the presence of Sr. The proportion in which K and Rb are taken up by the plant proves to be the same as in the solution. COLLANDER concludes from these data that the plant is able to make a selection in absorbing ions but that this selective mechanism cannot discriminate between closely related ions.

From this investigation, as well as from my own experiments, it appears, contrary to the opinion of HOAGLAND and coworkers, that ions which are absorbed by the plant do not influence each other unless they are closely related. The investigations by HOAGLAND and coworkers, corroborate these experiments since here, too, only closely related ions influenced each other.

From the investigation made by ARISZ (1944) as to the absorption of phosphate and various amino acids and asparagine by the tentacles of *Drosera capensis*, it also appears that the plant does, in fact, discriminate between phosphate on the one hand and amino acids plus asparagine on the other, but not between amino acids and asparagine. ARISZ concludes that phosphate ions are evidently bound to other spots of the plasm than are amphoions.

KROGH (1938) finds that there is also selectivity as regards the absorption of salt by various water animals, whilst the halogen ions can replace each other.

In addition to these direct data concerning the influence on various similarly charged ions during absorption, there is another source of data pointing in the same direction, viz. the experiments as to the exchange of ions already absorbed, and ions from the solution. Plants or tissues which have previously accumulated ions evidently lose practically nothing to distilled water: HOAGLAND and BROYER (1936); JENNY, OVERSTREET and AYERS (1939); BROYER and OVERSTREET (1940). HOAGLAND, HIBBARD and DAVIS (1926) established the fact that Nitella cells which have first absorbed Cl do not lose this to solutions with phosphate, nitrate or sulphate ions, but that they do so to solutions with Br ions. The reverse, the exchange of Br for Cl, also takes place. ROSENFELS finds the same for Br and Cl in the case of *Elodea*.

In the last few years it has become manifest that a similar phenomenon also occurs in experiments on the absorption and loss of radioactive ions. Thus, JENNY and OVERSTREET (1939) found that radioactive K absorbed by excised barley roots is exchanged for normal potassium. In distilled water however, or in solution with other cations, no loss of radioactive potassium takes place. BROYER

and OVERSTREET (1940) come to the same result. Here, it moreover appears that the exchange continues normally also at a temperature of 0°C. The active process of absorption, however, is at a standstill. OVERSTREET and JACOBSON (1946) made similar investigations for anions; barley roots at 0°C. exchange previously absorbed radioactive Rb and PO_4 for the inactive isotope, though this exchange proceeds more quickly for Rb than for phosphate. The writers surmise that the phosphate ions in the plant are more strongly bound than the rubidium ions.

From all these investigations it is manifest that the plant exchanges ions, once absorbed, only for isotopes or closely related ions.

An exception was found by JENNY, OVERSTREET and AYERS (1939) as regards the exchange of potassium. When, instead of solutions, certain clays entirely or partly saturated with a given cation were used, barley plants could lose absorbed radioactive K, also to Ca clay or Na clay. This stronger activity of clay suspensions is attributed by the writers to contact exchange of ions between the surface of the root and the clay. An investigation was made as to possible toxic activity of these clays. Though little of it was found (only hydrogenous clay was examined), it is nevertheless possible that the nature of the milieu was not very physiologic (thus, for instance the pH of hydrogenous clay is 2.45!) and that the exosmosis to clays saturated with one single definite non-related ion is the result.

However, by the side of these data, from which it is evident that similarly charged, but not closely related, ions do not influence each other in the absorption by the plant, there are numerous others where there is a question of influence. I have in mind the investigations into the antagonism of ions.

PIERRE and BOWER (1943) made a summary of the literature that has appeared on the influence of the mutual relations of cations on growth and absorption. Especially the influence of other ions in the K absorption is here dealt with. From this it appears that when only a single definite ion, in a fairly strong concentration is present in the medium of the plant, the growth of the plant, as well as the absorption of this ion, are strongly inhibited. This can be compensated by adding other cations to the solution. The so-called antagonistic effect between K and Ca is well known. Yet it is evident that the reaction on a given K/Ca relation is different for each individual plant. Similar phenomena as here described for cations have also been demonstrated for anions.

Thus HAMNER (1940) and MULLISON (1941) report that with soja and barley the growth is retarded when the plants are given phosphate, but no nitrate. On adding this latter ion an improvement

immediately sets in. Here, too, an effect poisonous to the plant is attributed to the one salt solution.

HAAS (1947) has shown that with citrus plants phosphate absorption from a solution not containing nitrate is much stronger than when nitrate is present. This proves that similarly charged, but not closely related, ions may influence each other in the absorption.

However, these experiments on antagomism of ions cannot be directly compared with the first mentioned experiments for the following reasons:

1. Fairly concentrated non-physiological solutions were used.
2. The duration of the experiment was usually much longer.

The result is that here a quite different situation arises than in my experiments. For a certain relation between the ions may, when this is non-physiological, cause changes in the plasm, from which, in its turn, a change results in the relative absorption of the various ions. The antagonistic activity of K and Ca with regard to the degree of swelling of the protoplasm is well known, STRUGGER (1935). Also a certain proportion of ions in the culture solution may influence the process of metabolism in the plant to such an extent that a modification in the proportion in which the ions are absorbed may be the result.

If therefore in the absorption process one wishes to study the mutual influence of the ions in the medium, experiments of short duration must be made in balanced solutions in order to exclude secondary and injurious activities.

The results of such experiments show, as we have seen, that only closely related ions influence each other in the absorption. I should therefore like to extend ARISZ' supposition that the protoplasm binds phosphate ions and amphoions in different places and transports them independently of each other, to all not closely related ions provided they are well absorbed by the plant.

§ 7. *Summary of the results.*

The results of the experiments mentioned in paragraphs one to five show that with regard to phosphate absorption there are important differences between low-salt and high-salt plants.

This is evident from:

1. The absorption as to time.

With high-salt plants this proceeds at a constant rate; with low-salt plants, on the other hand, the rate of absorption at first increases gradually and only after some days becomes constant (fig. 2 and 3). In most cases the rate of absorption by low-salt plants is considera-

bly higher than by high-salt plants (fig. 2 and 4); occasionally it happens that the rate of absorption by low-salt plants is at first lower than that by high-salt plants (fig. 3). These differences in rate of absorption are dependent on the condition of the plants.

2. The connection between absorption and concentration.

With high-salt as well as low-salt plants the absorption no longer increases above a certain concentration. The maximum rate of absorption is, with high-salt plants, reached at a much lower concentration than with low-salt plants (fig. 4).

3. The influence of light on the absorption.

With high-salt material good absorption requires exposure to light; in darkness the absorption comes to a complete standstill and, in most cases, passes into a slight loss. This loss is not the result of injury to the material (figs, 5, 6 and 7).

With low-salt plants a reasonable absorption is possible in darkness (table 4).

Besides these differences as to the rate of absorption others have been found between high-salt and low-salt plants, viz:

1. Differences in the rate of growth.

These differences correspond entirely with the differences in the rate of absorption. The curves indicating the process of the rate of absorption for both kinds of material run entirely parallel with those for growth (fig. 3). This was also found with high-salt plants when they were placed in darkness; simultaneously with the rate of absorption also the growth rapidly falls (fig. 6).

2. Differences in the percentage of carbo-hydrates.

In the roots of low-salt plants the percentage of total-sugar is about twice as high as in those of the high-salt plants (table 3).

The results of the experiments mentioned in par. 6 show that in experiments of short duration the absorption of phosphate by maize plants is not influenced by the presence of the other anions in the culture solution (table 5).

CHAPTER IV

DISCUSSION OF THE RESULTS.

§ 1. *Some observations on the process of absorption and transport of ions.*

In order that the results found may be better interpreted I will begin with a short exposition of the processes of absorption and transport of ions. For a more detailed treatment I refer my readers to the literature mentioned in the introduction.

Although the processes of absorption and transport can never be completely separated, a large number of investigators have used isolated cells or parenchymatic tissues so as to exclude at least transport over great distances, thus making the process less complicated. Also the experiments of HOAGLAND and coworkers with excised root systems relate to the absorption of ions in the cells of the root system itself, since here, too, no transport to the shoot takes place. If, however, intact higher plants are used, there is, along with the absorption of ions by the cells of the root, transport to the shoot. This transport, as is now generally accepted, takes place chiefly through the xylem. The ions in the xylem are supplied by the root, whereas the consumption takes place through the shoot.

I will first consider more closely the supply of ions by the root. An investigation into this matter was made by HOAGLAND and BROYER (1942) by which the observations of LUNDEGÅRDH (1940) that the ions are present in a higher concentration in the bleeding sap than in the outward solution could be confirmed. This signifies that the ions are carried to the xylem vessels against a concentration gradient and that for this transport energy is required. Indeed, roots in an N atmosphere proved to be no longer capable of accumulating ions in the bleeding sap.

In what way we must picture to ourselves this active transport through the root is not yet clear. So long as we cannot form a more definite idea of this we speak, after the example of HOAGLAND (1943) and ARISZ (1945), of an active secretion process responsible for this accumulation of ions in the xylem, without connecting it with more exact ideas as to the nature or place of the process. The factors which may then influence the secretion process are: The supply of oxygen, the supply of carbo-hydrates, the temperature and the concentration of the ions in the culture solution. The supply in the xylem will depend on the strength of the secretion process and the extent of the surface where the secretion takes place.

From an investigation by HOAGLAND and BROYER (1936) it appeared that excised low-salt barley roots were capable of accumulating ions (K and Br) in the root cells. This absorption, however, gradually decreases and comes fairly quickly to a standstill. In their opinion this results from the fact that in mature cells accumulation cannot continue to an unlimited extent. For with these cut roots no transport of ions to the shoot is possible, and also the growth will be of small account since the supply of carbo-hydrates has come to a stop. The cells of the root tissue at first contain few ions but in proportion as the accumulation increases the absorption decreases and eventually, when the cells have reached their maximum in ions, comes to a standstill. In fact it is evident that excised barley roots, whose cells have from the beginning a high percentage of ions, are entirely incapable of absorption. If, however, entire barley plants in high-salt and low-salt condition are examined (BROYER and HOAGLAND, 1943) it appears that, in both cases, a long continued absorption is possible, since ions can be transported to the shoot, and sugars from it.

Although this has not been investigated for the shoots separately, we may also here assume that there is a limit to the quantity of ions which mature cells can contain. When we consider the consumption of ions by these parts of the plant, then, in all probability, the same factors will be of influence as is the case in the absorption of ions by the root from the outer solution. Here, the absorption will be dependent on the concentration of ions in the xylem, the supply of oxygen and carbo-hydrates and the temperature. It is known that also light influences the absorption; in what manner I will leave for the present undecided. As here, too, the number of ions that can be fixed by the shoot is limited, the absorption and the growth will in a high degree be connected. The total consumption will, also here, depend on the strength of the accumulation mechanism and on the surface where this mechanism is active.

Now that I have discussed the processes of supply and removal in the xylem, we can distinguish between two possibilities: either the capacity of the shoot to fix ions is greater than the supply by the root or it is smaller.

If the capacity of the shoot is greater than the supply, all that is transported through the xylem can be fixed by the shoot, and it is possible that the rate of growth of the shoot is determined by the speed with which the supply of ions takes place.

It is also possible that the capacity of the shoot is less than the supply in the xylem. In that case there will be a storage of ions in the shoot unless the plant is in some way or other capable of

avoiding this. Of some plants (e.g. various halophytes) it is known that they can excrete superfluous salts through the leaves, so that in this manner accumulation is avoided. This mechanism, however, does not take place in all plants and certainly not in the maize plants which I used. It was only during the first twenty four hours that guttation was observed with the low-salt plants. The possibility exists that along with this exudation phosphates from the xylem are excreted, but for the rest of the duration of the experiment the storage is evidently prevented in some other way.

We can imagine two possibilities whereby the storage of salts in the xylem is avoided.

1. The terminal shoot can regulate the salt secretion of the root in the xylem according to consumption.
2. The superfluous ions are transported back to the root by other channels.

The first-mentioned possibility is very improbable, for the xylem vessels are non-living elements so that direct regulation can here not very well be assumed. It might be possible to imagine that the concentration gradient between root tissue and xylem sap could influence the intensity of the secretion, but neither is this plausible since the secreted ions are carried upwards by the transpiration flow. The investigations of BROYER and HOAGLAND (1943) show that a stronger transpiration has, in the case of low-salt barley plants, no influence on the uptake of K and Br, only that the ions are more rapidly transported through the xylem vessels. With high-salt plants on the contrary there is an influence of transpiration on the uptake, but, as the differences found are correlated with differences in fresh-weight, these latter differences may be the direct cause. Therefore BROYER and HOAGLAND conclude that transpiration may have an influence on the rate of transport through the xylem. It has apparently no influence on the secretion mechanism in the root.

There are, however, various indications pointing to the second possibility. STOUT and HOAGLAND (1939) demonstrated that in the shoot ions can move to the bark in a lateral direction. MASON and coworkers (1931, 1937, 1940 a b) observed that if a ring is cut in the bark, certain ions (K, Mg, Cl, and P) together with carbo-hydrate and nitrogen compounds accumulate above the ring. MASON and PHYLLIS (1937) surmise that such a downward transport through the phloem depends on the concentration gradient. They assume that there can be a circulation of ions in the plant, so that ions carried upwards through the xylem along with the flow of water can, in case of inadequate consumption by the shoot, return to the root through the phloem.

A similar circulation is assumed by COLLANDER (1941). With a constant secretion from the root into the xylem the absorption of ions from the outer solution may, in this case, decrease because the plasm of the root cells also receives ions from the shoot. Thus the consumption of the shoot will influence the absorption by the root.

§ 2. *The differences between low-salt and high-salt plants.*

We have already seen that HOAGLAND and coworkers found for low-salt and high-salt barley roots that the expressed sap of the former contains more carbo-hydrates and less ions than that of the latter. The low-salt roots may, for a short time, have a strong capacity for accumulation when they are placed in a salt solution whereby according to HOAGLAND the sugars in the vacuole are partly replaced by ions. The excused high-salt roots are not capable of accumulating ions. Entire high-salt plants with which a transport to the shoots is possible are, on the other hand, able to absorb ions. Moreover, these plants are also capable of transporting carbo-hydrates from the shoot to the root. As regards the high-salt and low-salt maize plants which I used, we know that the percentage of carbo-hydrates in the roots of the low-salt plants is considerably higher than that of the high-salt plants, but there was no test made as to a possible difference in the salt percentage. When these two kinds of material are placed under the same conditions in which they are able to absorb ions, then we see that the low-salt plants usually absorb a much greater amount of phosphate than the high-salt plants, and have a more vigorous growth.

In connection with the investigation by HOAGLAND and BROYER (1936) and what was stated in the previous paragraph, we may assert that the phosphate absorption by the low-salt plants may be greater than that by the high-salt ones for two reasons:

1. Accumulation of ions is still possible in the mature cells of the plant.
2. The growth of the low-salt plants is stronger than that of the high-salt plants.

As to the first point I have no data at my disposal concerning the maize plants which I used. With regard to point 2, I found that with low-salt plants there is a stronger growth and a higher percentage of carbo-hydrates than with high-salt plants. In discussing the results I will confine myself as much as possible to these data, but I consider it probable that the differences are also due to differences in the percentage of phosphates of the mature cells.

In the previous paragraph it has been made clear that an increase in the percentage of carbo-hydrates may result in a stronger secretion

into the xylem, as well as in an increased consumption of ions by the shoot as a consequence of stronger growth. Therefore, though in my experiments we find a clear connection between absorption and growth, it must still be ascertained whether the growth determines the absorption or vice versa. For this is dependent on the question as to what is the limiting factor. If the capacity of the shoot for absorbing phosphate is greater than the supply by the xylem, then an increased supply of carbo-hydrates may result in an increased supply of ions by the roots, and, secondarily, in a vigorous growth of the shoot; if, on the other hand, the supply is abundant enough to cover the consumption of phosphates, an increased supply of carbo-hydrates may primarily result in a stronger growth, and secondarily in an increased absorption. In the ensuing paragraphs (3 and 4) we shall see that it is indeed possible to demonstrate that in a given case the change in growth is primary, and determines the degree of absorption of phosphate.

Besides these quantitative differences in the rate of absorption and growth there are also qualitative differences between the two kinds of material. Under constant conditions both growth and absorption show, at first, an increase with the low-salt plants which is not the case with the high-salt plants (see fig. 3). It seems obvious that this should be connected with the difference in the percentage of carbo-hydrates of the roots. According to HOAGLAND the carbo-hydrates in the vacuoles of the roots of low-salt plants are replaced by ions when the plants have an opportunity to absorb them.

It is quite conceivable that also in my experiments (figs. 2 and 3) the increase in absorption and growth with low-salt plants during the first few days of the experiment is a result of this replacement of the carbo-hydrates in the vacuole, so that gradually more carbo-hydrates are liberated for the processes of metabolism. This means that at the beginning of the experiment the supply of carbo-hydrates is the limiting factor in the processes of salt absorption and growth.

It is, however, to be expected that when a low-salt plant is given the opportunity of absorbing ions, it will gradually pass into a high-salt condition, that is, that the extra quantity of carbo-hydrates is consumed and that the accumulation of ions by mature cells comes to a standstill. In the course of time this would again result in a decrease of the rate of absorption. From fig. 2 it appears that such is not the case and that also with the low-salt plants the absorption can continue, at least for some days, at a constant rate. This is understandable when we assume that at first the supply of carbo-hydrates has a limiting effect on the absorption, but that very soon the carbo-hydrates essential for absorption and growth are present in excess

and that another factor becomes limiting. In any case it appears from table 4 that with the low-salt plants the supply of carbo-hydrates has no limiting effect since, in darkness, absorption and growth continue, whereas the formation of carbo-hydrates comes to a stop.

§ 3. *The influence of light.*

The investigation concerning growth and absorption in light and in darkness showed that in the case of high-salt plants both come to a standstill in darkness, but that with low-salt plants they continue. The fact that the growth of high-salt plants comes to a stop cannot be a result of deficiency of ions, because in the cultivation of the material it was clearly demonstrated that with high-salt plants growth takes place when they are put on tap water in order to bring them to low-salt condition. In this case absorption of ions is therefore not possible, and from the fact that nevertheless there is growth it is evident that the percentage of ions in the plants themselves is sufficient to allow it to continue. Now, when in darkness a standstill in growth and absorption is reached, it is evident from the above that the stopping of the growth results in a standstill in the absorption and not the reverse. Darkness, therefore, causes a standstill in the growth of high-salt plants. With low-salt plants this is not the case, and the influence of light proves therefore to depend on the condition of the material.

The only distinct difference which I found between high-salt and low-salt plants is the percentage of carbo-hydrates in the roots. There is a possibility that the standstill in growth of the high-salt plants in darkness is the result of a deficiency of carbo-hydrates.

WENT and BONNER, too, found (1943) that, in darkness, the growth of tomato plants is greatly diminished, and they also surmised that this was caused by a deficiency of carbo-hydrates. In fact, it appeared that the growth could be made to be resumed in darkness by administering sucrose to the leaves.

I tried to verify this hypothesis by using the same method, but the result was negative (cf. chapter 3 p 38).

Now it further appeared from the investigation of WENT and BONNER that the growth of their tomato plants did not depend only on the supply of carbo-hydrates but also on other factors, viz: the supply of growth hormones and of a substance which they named caulocaline and which is formed in the roots.

It is therefore possible that in my experiments it is not the deficiency of carbo-hydrates that stops the growth of high-salt plants, but that still other differences exist between high-salt and low-salt

plants to which can be attributed the difference in behaviour in darkness.

The influence of light here demonstrated is indirect i.e. exposure to light of the shoot influences the absorption of phosphate by the root. A similar influence of light was also demonstrated by SCHMIDT and by LUTTKUS and BÖTTICHER. Here, too, the exposure to light of the shoot proved to have a strong influence on the uptake of salt by the root, but it appeared that this influence was not the same for the ions examined. It is strongest for K whilst for Ca scarcely any trace of influence was found. This points to the fact that there is no question of only stronger growth by exposure to light, since, in that case, it may be assumed that light would influence the absorption of various ions in the same degree. I do not know of any closer investigation into the nature of this influence of light. LUTTKUS and BÖTTICHER present as a hypothesis that in light K can be bound to the plasm but that this binding is broken again in darkness. SCHMIDT assumes that K plays a part in the process of photosynthesis. In paragraph five I will revert to these hypotheses.

Along with the investigation concerning the influence of light on intact higher plants, also less complicated objects have been used to work with. HOAGLAND, HIBBARD and DAVIS find that the Br absorption by *Nitella* cells is greater in light than in darkness; INGOLD finds the same for the absorption of K, Cl and P with terminal shoots of *Elodea*. Nor was there here a closer investigation made; in both cases the authors surmise that light acts as a source of energy in the absorption. Only ARISZ (1947) made a closer inquiry into the nature of the influence of light, and he found that the intensifying effect of light on the absorption of Cl by *Vallisneria* leaves remains undiminished when the milieu is free of CO_2.

Thus from my experiments it merely became clear that the standstill of phosphate absorption in darkness which I found with high-salt plants, is a result of the stand-still of the growth, and that therefore, in this case, the growth has a restrictive effect on the absorption.

However, in what manner light influences the growth and, at the same time, the absorption, has not been settled by my experiments.

§ 4. *The influence of the concentration on the absorption.*

Besides the influence of light, also that of the phosphate concentration of the solution on the phosphate absorption was examined.

When discussing the investigation by VAN DEN HONERT we saw that according to his supposition the concentration whereby a maximum absorption is reached for a definite object, always has a

definite value. I did not arrive at the same result; for this value was for high-salt plants 12 mg P_2O_5/l and for low-salt plants 24 mg P_2O_5/l. If we attempt to explain this we must go somewhat further into the theoretical conceptions of the process of ion absorption.

As I mentioned already when discussing the literature on these experiments in Chapter III paragraph 3, it is universally acknowledged that an adsorption process is linked up with the absorption. From the experiments concerning the influence of various anions on the phosphate absorption (Chapter III par. 6) I concluded that the phosphate ions are bound to definite points of the protoplasm. Now, when the connection between absorption and concentration points to an adsorption process, this means that the spots in the plasm which are capable of binding phosphate ions are not all occupied by them when there is a low concentration of the outer solution. With an increasing concentration more points are occupied until, at a certain intensity, phosphate ions are bound to all the available spots. By the secretion mechanism these ions are in some manner liberated and given up to the xylem. The spots in the plasm which become vacant will eventually be replaced by ions from the solution. If only a small number of spots are occupied then only few ions will be secreted into the xylem in a given unit of time; according as more ions are adsorbed the rate of secretion accelerates until, in the end, with a maximum adsorption the maximum secretion is reached. A futher increase in the concentration has then no longer any effect. The secretion into the vessels of the xylem being an active process is, as well as on the degree of adsorption, also dependent on the supply of oxygen and carbo-hydrates and on the temperature.

VAN DEN HONERT (1933) conceived a model by which this process is illustrated. According to this conception, which was first made by VAN DER WEY (1932) in connection with the transport of auxin, the ions are conveyed on a belt running inwards from the root surface. With a low concentration of the outer solution, this belt is only partially loaded; with increasing concentration the load increases until, at a certain intensity, it reaches its maximum. The rate of absorption depends not only on the load, but also on the speed at which the belt rotates. This speed is influenced by the temperature, a factor examined by VAN DEN HONERT, and also by the supply of oxygen and carbo-hydrates. The concentration at which the adsorption on the plasm is maximal is, according to VAN DEN HONERT, constant for all plants (cf. Chapter III par. 2), because it is determined by the properties of the plasm. I made my experiments with material which differed in the percentage of carbo-hydrates and probably also in ion content. Moreover, it is possible that the size

of the root surface of the low-salt plants differed somewhat from that of the high-salt plants. These differences find expression in a difference in the rate of absorption and growth.

Seen in the light of the above-mentioned model, the percentage of carbo-hydrates cannot be of any direct influence, for a difference herein might cause the process of secretion to work more quickly or, according to the conception of VAN DEN HONERT, cause the conveyor belt to rotate more rapidly, but the adsorption equilibrium remains unaffected. The same is the case when there is a difference in accumulation by mature cells or a difference in root surface. Also here, the only result is, that the region in which the secretion process is active differs (a difference as to the number of conveyor belts) but the position of the equilibrium remains the same in both cases. These differences can, therefore only explain the fact that the rate of absorption by low-salt plants is, with all concentrations, greater than that of high-salt plants.

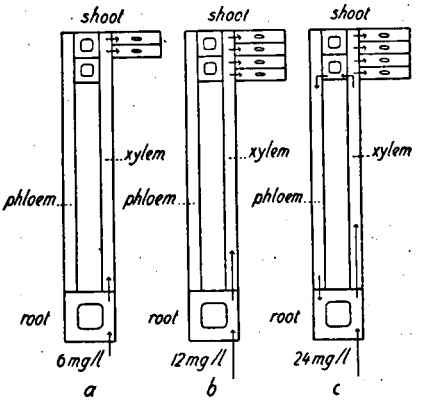

Fig. 8. Schematic representation of the process of absorption and transport of ions by high-salt plants with different concentrations of the culture solution.

In the previous paragraph I mentioned already that the absorption by high-salt plants is probably determined by the growth of the shoot, so that in this case, the capacity for fixing ions is smaller than the supply of ions by the root. According to the conception in paragraph 1 this can be explained by assuming that a part of the ions which get into the stem are conveyed back to the root via the phloem. These ions may then once again, by means of the secretion mechanism, be excreted into the xylem vessels i.e. they can take part in the loading of the conveyor belt. As can be seen in fig. 4 the rate of absorption of phosphate no longer increases by a concentration in the solution higher than 12 mg/l. The secretion into the xylem, however, may then increase with both high-salt and low-salt plants. The fact that the absorption from the outer solution increases with low-salt plants and not with high-salt ones must be the result of the conveyor belt being, in the case of high-salt plants, partly loaded with ions which return from the shoot.

The course of the process is shown in fig. 8. The root tissue is here represented by a single cell which is connected with the shoot by a phloem vessel and a xylem vessel. The shoot is represented by a few mature cells between the xylem and the phloem, and a few growing cells. The number of these growing cells is a measure for the rate of growth. The length of the arrows in the figure indicates the extent of the absorption, respectively the secretion. In fig. 8a the situation is given with a low concentration of the outer solution. Here, the spots in the plasm that convey the ions are few and therefore there is little secretion and consequently little absorption. In fig. 8b the concentration of 12 mg/l is reached; secretion and absorption have increased and, as a result, the growth has become stronger and has reached the maximum. If the concentration becomes still more intense as in fig. 8c the secretion increases on account of a greater adsorption on the plasm, but the growth does not become greater. The surplus of ions is conveyed back to the root through the phloem, and the absorption from the outer solution is, in this case, equal to that in b and is, therefore, less than the secretion into the xylem.

This representation is, certainly, a schematic one and the number of data on which it is based comparatively small. I have given it here because it is in line with the now prevailing conceptions as to the processes of absorption and transport, and because it demonstrates what a complicated course the process of ion absorption may take with intact higher plants.

§ 5. *Selectivity of absorption and exosmosis.*

The experiments as to the influence of the concentration of the accompanying ions on the absorption of phosphate lie somewhat outside the frame of the results discussed in the previous paragraphs and I will, therefore, deal with them separately.

The fact that the absorption of phosphate proceeds independently of the concentration of the other ions, caused me to surmise that different anions are bound to different points of the plasm and transported independently of each other. The selectivity which we find in the absorption (cf. COLLANDER 1937 and ARISZ' plasm binding theory 1944) might be based on the fact that the protoplasm, by the nature and the number of the binding possibilities it possesses, determines what ions can be actively taken up and in what proportion. Little can yet be said about the nature of this binding. However, its great specificity suggests that the ions are not transported through the plasm without having any further definite object but that specially those ions are transported which have a function in the plant

metabolism. Thus PIRSON (1939) finds that K has a function in the process of photosynthesis and that here it may, at least partly, be replaced by Rb. COLLANDER (1941)finds, as we have seen, that the same applies to the absorption. Here, too, the plant makes no distinction between K and Rb.

This conception viz. that the ions have a function in the processes of metabolism and that, therefore, absorption and metabolism are much more closely connected than was at first supposed has, during the last few years, come more and more into prominence: HÖBER (1940), HOAGLAND and BROYER (1942), JACOBSON and OVERSTREET (1947), STEWARD and STREET (1947). Thus HOAGLAND and BROYER declare "The general picture would seem to be that permeability, metabolism, and accumulation of a salt are so intimately interrelated that generally it becomes impossible to disentangle the several aspects of the phemomena". (Page 879). JACOBSON and OVERSTREET, also, find that ".... it is almost essential to invoke a chemical mechanism in order to explain the intimate relationship between aerobic metabolism and accumulation". (Page 419). We find the same ideas with STEWARD and STREET who, in their recent survey of the absorption of salt, also distinctly stress the coupling of the processes of salt absorption and metabolism. I will here give a quotation: ".... the major processes of plant metabolism and nutrition — respiration, photosynthesis, absorption and accumulation of salts, intake of water — were for long treated as discrete processes". (Page 471) and "No longer, however can respiration be treated as though it concerned carbo-hydrate breakdown alone. It is the interrelations between nitrogen metabolism in plants and other vital processes which constitute a salient problem today". (Page 472).

Thus, the process of ion absorption is no longer looked upon as a result of plant metabolism but rather as being part of this metabolism. The quantity in which a certain ion can be bound by the plasm will therefore depend on the activity of the latter. In this connection it is interesting to note once more the hypothesis of LUTTKUS and BÖTTICHER. As we have seen in paragraph three these writers surmise that, in light, potassium ions are bound to definite elements of the plasm, but that in darkness this binding is broken, in consequence of which, in darkness, the potassium is lost to the solution. Now some investigators have already supposed that K plays a part in photosynthesis. SCHMIDT (1936) arrived at this opinion because in light the K absorption is increased to a very high degree; PIRSON (1939) found that the photosynthesis of Chlorella cells, deficient in K, increases greatly by the addition of K. The hypothesis of LUTTKUS and BÖTTICHER becomes more understandable

when we presume that in light the potassium ion is bound to certain intermediate products in the photosynthetic process. If these products are present there is an increase in what I may call the binding capacity of the plasm; in darkness, however, these intermediate products are assimilated and the binding capacity once more diminishes. By binding capacity of the plasm for a certain ion I, therefore, mean the quantity of that ion which under certain conditions may be bound to the plasm.

WIERSUM (1947) too, introduced a similar idea. By „accumulation level" he understands the number of ions present in the plasm, either free or bound.

In order to avoid confusion with the word 'accumulation', which usually refers to the heaping up of ions in the vacuole, I should prefer not to borrow this term. Moreover, the two conceptions do not completely cover each other, since WIERSUM includes all the ions present in the plasm, whereas I take into account only those which are bound.

Thus, I therefore surmise that the binding of ions is very closely related to the part which ions play in metabolism. I cannot give a more precise definition of this binding; we are, however, compelled to assume that this binding is either very labile or of short duration. For, from the experiments of JENNY and OVERSTREET (1939) and JENNY, OVERSTREET and AYERS (1939) it has become evident that the ions present in the plasm are continuously exchanged for ions from the outer solution (cf. Chapter III paragraph 6). BROYER and OVERSTREET (1940) demonstrated a probability that this exchange is connected with the ions present in the plasm. The fact that this exchange continues normally at a temperature of 0°C, at which the processes of metabolism practically come to a standstill, might indicate that we have here to do with a rather labile binding. Moreover, from the fact that the ions can be accumulated in the vacuole and, in the case of roots, can be conveyed to the xylem, it appears that the binding to the plasm can only be a temporary one.

In paragraph one I have assumed with HOAGLAND and ARISZ that, in the latter case, by means of a still unknown secretion mechanism, the ions are freed from the binding to the plasm and given up to the vessels.

In my opinion, it is necessary to assume some such active mechanism, since the transport of ions from the outer solution to the plasm takes place against a concentration gradient.

The necessity of postulating this secretion process has not always been felt. Thus CRAFTS and BROYER (1939) tried to explain a polar transport in the root by assuming that there is a difference in the

supply of oxygen between the cortex and the central cylinder. As a result, the cortical cells can absorb more ions than the cells of the central cylinder. This difference in the percentage of ions is said to be the cause of a movement of ions from the outside to the inside through the symplast. Within the endodermis, however, the protoplasm can no longer bind the ions which then, as free ions, are present in a high concentration in the tissue inside the endodermis. As a consequence, water is sucked in from without; pressure is exercised within the endodermis, by which the ions along with the water — this being the way of least resistance — are pressed upwards through the xylem vessels. As a matter of fact, WIERSUM (1947) is of the same opinion; however, he speaks of a difference in — "accumulation level" between cortex and central cylinder and expressly excludes from transportation, ions in the vacuole.

In both conceptions it is not clear what is the force that causes the ions to move from the cortex to the central cylinder. CRAFTS and BROYER speak of diffusion and flow of protoplasm. But when, as is generally accepted, we assume that the majority of the ions present in the plasm are bound, and that at least the free ions present are only temporarily free, there is no reason to assume that there is a concentration gradient between cortex and central cylinder. Nor is there in WIERSUM'S representation any reason why the ions should move from places with a high accumulation level to places with a low accumulation level. In my opinion, a directed movement in the plasm occurs when somewhere there is a deficiency of ions. This deficiency can arise in various ways viz. on account of the growth i. e through the formation of new plasm and the fixing of ions in organic elements (N, P) and through the removal of ions from the plasm by secretion into the vacuole and into the xylem vessels (root).

In conclusion, I must mention that in my opinion, it is not necessary to assume that only in the above-mentioned manner can the ions be absorbed by and transported through the plasm. Also, the ions which are not essential for the plant, such as Na, can be absorbed. This absorption, however, will then for the greater part be based on a passive penetration.

SUMMARY.

It has long been known that the supply of ions has an influence on the growth of the plant, in the sense that with a deficiency of ions the growth is hampered. The connection between absorption and growth has, in this treatise, been more closely investigated with plants of different salt percentages by comparing the rates of absorption and growth and by studying the influence on these of some external factors.

In order to obtain the material suitable for these experiments seed plants of Zea mais L. were cultivated on a HOAGLAND nutrient solution of half strength, supplemented with A-Z solution. Phosphate and iron were alternately administered, by which chlorosis could be avoided. By placing part of the plants on tap water some time before the experiment, and regularly providing others with fresh nutrient solution, plants with a low percentage of salt (low-salt plants) were obtained in the former case, and plants with a high percentage of salt (high-salt plants) in the latter.

Some experiments were made with an arrangement for continuous flow; in the other cases the absorption was determined from solutions without a continuous flow. Advantages and disadvantages of the method of continuous flow have been fully discussed in Chapter II. In every case, the absorption was determined by analysis of the outer solution.

From the results it appeared that with both kinds of plants the absorption can continue for several days. With the *high-salt* plants, the rate of absorption remains the same under constant conditions; with the *low-salt* plants it shows an increase during the first two or three days of the experiment, and only afterwards does it become constant. As a rule, the rate of absorption with low-salt plants is greater than with high-salt plants, but these differences are dependent on the external conditions during the cultivation.

Also with regard to the connection between absorption and *concentration* there are evidently considerable differences between low-salt and high-salt plants. This connection, it is true, has in both cases been shown by a saturation curve which is in accordance with what other writers have found in this respect, but the concentration at which the maximal rate of absorption is reached is much lower for high-salt plants than for low-salt ones.

Also with regard to the factor *light* there are differences between the two categories of plants. In darkness the absorption comes to a standstill with the high-salt plants and, in most cases, even passes

into an exosmosis; low-salt plants, on the other hand, are capable of absorbing phosphate in darkness.

In certain experiments the rate of *growth* was determined in addition to the rate of absorption. There always proved to be a close connection between absorption and growth. This is clearly shown from the experiments with high-salt and low-salt material under constant conditions. Here, in both cases, the growth curves run entirely parallel to the curves showing the rate of absorption i.e. with the low-salt plants also the rate of growth shows at first an increase, whereas with the high-salt plants this rate of growth remains on the same level during the whole length of the experiment. This connection proves also to exist with regard to the absorption in darkness. With high-salt plants both absorption and growth come to a stop whereas in an experiment with low-salt plants in darkness there was growth as well as absorption of phosphate. The differences in absorption between the two kinds of material are, therefore, clearly connected with differences in growth.

In accordance with experiments by HOAGLAND and BROYER the percentage of *carbo-hydrates* in the roots of low-salt plants proved to be considerably higher than that in the roots of high-salt plants. I surmise that with low-salt plants, the initial increase in absorption and in growth under constant conditions must be connected with increased metabolism in the roots of the low-salt plants.

From the experiments concerning the influence of light and that of the concentration on the absorption it may be deduced that in these experiments *the growth of the shoot is determinative for the absorption* with high-salt plants. This regulating influence of the shoot on the absorption by the root is explained by the hypothesis that, with high-salt plants a part of the ions which are secreted by the root in the xylem are carried back to the root by the phloem when the consumption by the shoot is smaller than the supply by the root. This is in accordance with the experiments by MASON and his co-workers, who have demonstrated a downward transport of ions through the phloem.

Finally, it was found in some experiments of short duration that the absorption of phosphate is, with both high-salt and low-salt plants, independent of the *other anions* in the culture solution. In agreement with other writers I assumed that the actively absorbed ions play a part in metabolism, and that the amount to which ions can be bound by the plasm (*binding capacity of the plasm*) is dependent on the metabolism. This binding is considered to be specific for a definite ion, in the sense that it can only be replaced by an isotope or a chemically closely related ion.

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