ANALYSIS OF THE PROCESS OF ANION UPTAKE OF INTACT MAIZE PLANTS

BY

R. J. HELDER

(Botanical Laboratory, Groningen, Holland)

Received June 15th, 1952

CONTENTS

I. INTRODUCTION

During the last years reviews of the ever increasing number of investigations into the salt absorption of plants or parts of plants appear regularly (LUNDEGÅRDH 1947; BURSTRÖM 1951; ROBERTSON 1950, 1951), so a few general remarks may suffice. It appears from the literature reviewed that a division of the absorption process according to physiological principles such as penetration into the cytoplasm or accumulation in the vacuoles is seldom made. On the other hand the analysis has been carried through so far already that physico-chemical and biochemical processes and factors take up a great place in the theoretical considerations as to the different mechanisms involved. The relationship between the salt absorption and the anion-respiration has been studied particularly and discussed thoroughly (LUNDEGÅRDH; ROBERTSON and collaborators). For the sake of simplicity many experiments have been performed with pieces of tissue (discs of storage tissue, root-pieces, resp. excised root systems). Remarkable and for methodical reasons explainable is the use of ions like bromide, chloride and rubidium. It is clear that some care should be taken in drawing conclusions from results obtained with these ions. This also

applies to founding hypotheses as to the absorption mechanism. Since uptake and metabolism appear to be intimately connected, the extent to which ions are assimilated by metabolism can be expected to influence the way in which they are absorbed.

As was already stated by ALBERDA (1948), the uptake by intact plants has as yet been studied very little. The experiments described here are a direct continuation of Alberda's. Intact maize plants were used. Mainly the uptake of phosphate was traced but occasionally the nitrogen absorption was traced. Unfortunately chloride was hardly taken up at all.

At the starting point were two problems. First the course of the uptake of phosphate and growth with low salt and high salt plants was to be determined. To be able to explain the course of uptake was the idea governing the planning of a series of experiments concerning the significance of sugar supply and growth for the absorption. Secondly, the relation between phosphate uptake and outer concentration of phosphate was studied. The problem as to what may be the nature of the first phase of the absorption and what is the cause of the well-known "saturation-phenomenon" will appear, taking an important place.

In describing the experiments, they are arranged according to a line of thought indicated below. At the end of every set of experiments a preliminary discussion will be given. By doing so, the general discussion can be restricted to the discussion of main principles and the forming of a preliminary picture of the salt absorption by intact plants.

An important question is, how far the root is permeable to the anions. The data concerning the loss of anions by the root is of interest in this respect. Intimately connected with this problem is the question of the relation of uptake and metabolism. The extent to which the metabolism plays a part, also in the very first phase of the absorption process, may be deduced from the experiments over the influence of phloridzin, sugar supply and ion-concentration of the medium.

It will appear that other processes must be assumed to have an effect on the uptake, in order to be able to explain the effect of the addition of glucose and of the "saturation-phenomenon". The correctness of this assumption will appear from the experiments about the relation between growth and uptake.

Finally an attempt must be made to explain the behaviour of the uptake in light and darkness with the aid of the data obtained in the meantime.

The experiments were performed between 1948 and 1951 at the Botanical Laboratory, Groningen. I am greatly indebted to Prof. Dr W. H. ARISZ for his constant interest and stimulating criticism. I also owe many thanks to Prof. Dr J. J. HERMANS for the instructive discussions on the theoretical aspects of the relation between concentration and uptake. Finally, I wish to thank Miss D. L. TOLMIE B. Sc. [London] for her correction of the English text.

II. MATERIAL AND METHODS

In all the experiments maize plants were used. Grains were soaked for two days and then allowed to germinate. When the coleoptiles were 3—5 cm long, the seedlings were mounted on wooden discs (11 cm diameter). In these discs were four holes. Two or three seedlings were fixed into every hole, making sets of 8—12 plants. They were grown for about four weeks on a half-strength Hoagland solution. The solution was always made with tap water. Especially at the start and at the end of the season some chlorose frequently occurred. To avoid this as far as possible, the sets were put on a minus-phosphorus solution with iron during the first week; then on a complete nutrient solution without iron. (OLSEN 1938 a, b). Better results were obtained by extending the minus-phosphorus, plus-iron period (1950). During 1951 an attempt was made to avoid chlorose by lowering the p_H of the solutions by adding diluted sulphuric acid. This provided the best results (FRANCO and LOOMIS 1947). A full-strength complete Hoagland solution could now be used without any trouble.

High salt material was obtained by renewing the solution twice a week. Especially in experiments in the middle of the summer (when the plants show a vigorous and rapid development) it appeared that the plants did not attain an extreme high salt condition. Therefore, in some experiments (1950, 1951) the solutions were changed daily just before the experiment, the plants being already in the room at constant temperature.

With low salt plants the solutions were no longer renewed after the first week. The experiments of 1948 showed that there was only a quantitative difference between the high salt and low salt plants as to the course of the uptake (exp. 6a; fig. 10). Therefore, the low salt treatment was intensified by putting the plants only once on a minus-phosphorus nutrient solution with iron. It was necessary to replenish the solutions frequently with tap water. A drawback of this last method was the inhibition of the development of the low salt material, particularly of the shoots. The rate of uptake by this material was less than that of the high salt. Because of this during 1950 and 1951 this extreme treatment was given up. It was attempted to make the high salt material as saturated as possible.

As a rule the sets were used four weeks after their having been mounted on the discs. The condition of the material at that moment was determined by the treatment and the circumstances as indicated above. Therefore, if necessary, with the description of the experiments details of these two factors will be entered into. However, to avoid any misunderstanding, we will speak of high salt plants, if the nutrient solutions were renewed frequently, and of low salt plants if this was not the case any longer after the first week. In some experiments nutrient solutions were used of a rather different composition or concentration. During the pretreatment as well as during the experiments, this sometimes happened. The p_H of these various solutions was always adjusted to 6·4—6·5. The nutrient solution used was as a

matter of fact poorly buffered between p_H 3·2—6·1. Above these values there was a reasonable buffering capacity. In spite of this the p_H always rose as a result of the absorption. After absorption periods of long duration values of 7·0—7·4 were attained. Neither iron nor A—Z solution was present in the solutions, used during the experiments.

The experiments were performed in a dark room at constant temperature (21° C.). Figure 1 shows some details of the arrange-

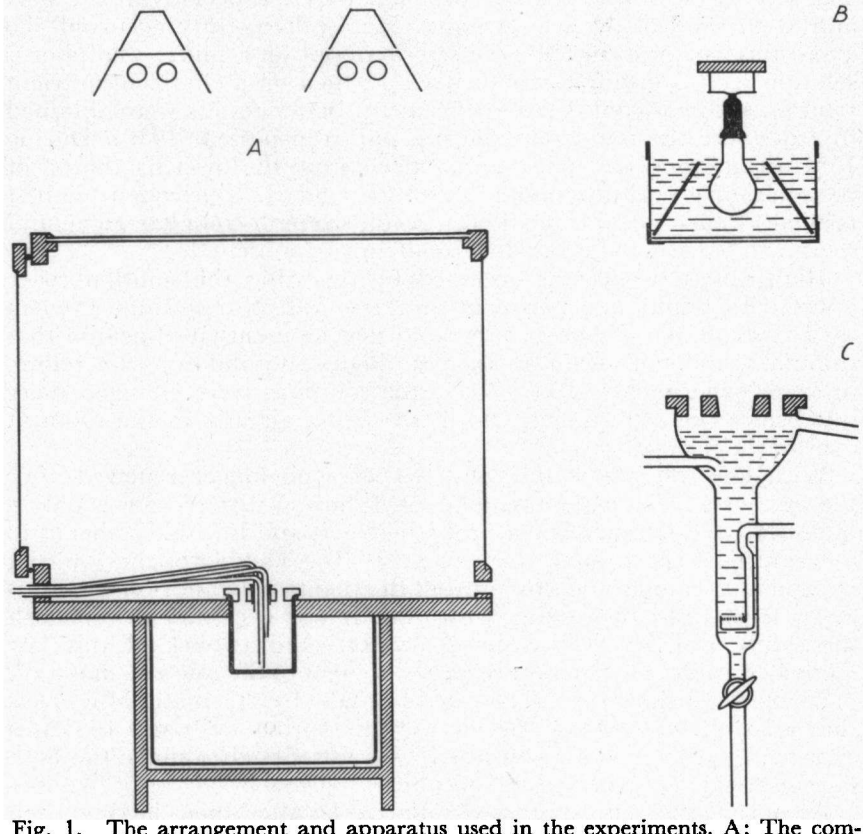

Fig. 1. The arrangement and apparatus used in the experiments. A: The complete arrangement with illumination by fluorescent lamps. Normal or carbon dioxide free air can be led through the case. B: The illumination by incandescent lamps, placed in running tap water. C: Root-chamber used in experiments with short absorption periods.

ment and apparatus used. In the experiments with absorption periods of 12—24 hours the sets of plants were placed on enamelled jars of rather more than one litre capacity; eight jars being fastened in a shelf (130 × 80 cm) and placed in a watertank. Moreover, on the shelf a case was constructed with a glass top and aluminium sides, allowing a good illumination. There were two compartments, which

could be hermetically sealed. In this way it was possible to lead through either normal or carbon dioxide-free air (fig. 1: A).

Originally the plants were illuminated by four fluorescent lamps at a distance of 70 cm above the jars. The intensity of these lamps was rather low (5.000 lux at most). Therefore, they were replaced at an early stage by incandescent lamps. Above each set of plants one lamp of 200 Watt was mounted. In order to carry away the heat produced by the lamps by means of running tap water, they were placed four and four in two small tanks with glass bottoms (fig. 1: B). There was at both sides of the lamps a strip of mirror glass. With this light source a maximum light intensity was attained of 16.000 lux; at a distance of 70 cm 2.000 lux, the plants being illuminated by an average light intensity of 9.000 lux.

The sets of plants were always placed on one litre of a nutrient solution that was moderately aerated. The solutions were drained off by careful siphoning every 12 or 24 hours. In experiments with absorption periods of 1—4 hours a root-chamber was used as is illustrated by fig. 1: C. It was a part of an arrangement as used by WOODFORD and GREGORY (1948) and VAN ANDEL, ARISZ and HELDER (1950). It allowed a rapid changing of the solutions. Moreover, the roots could even be rinsed by distilled water within a few minutes. This is important to those experiments in which solutions of various composition were used.

The rate of uptake was determined by analysing the medium at the beginning and at the end of the absorption period. In most experiments phosphate uptake was determined, in some experiments nitrate was, and occasionally both substances were. Colorimetric methods were applied: phosphate according to the molybdenum-blue method (PARKER and FUDGE 1927), nitrate with the phenol disulphonic acid method (SNELL and SNELL 1936).

In a few experiments the nitrate content of the solution had to be determined in the presence of organic matter. The colorimetric method was less suited to this. In this case samples of 10 cm^3 of the solution were carefully evaporated to dryness. The total nitrogen content of the residue was determined according to the micro-Kjeldahl method (LOOMIS and SHULL 1937). The results remained rather variable. By applying both methods to the same solution without organic substances, the Kjeldahl method proved to give higher absorption values than that of the phenol disulphonic acid method.

Plant material of the experiment of 1949 was also analysed. The root systems were excised, rapidly dried with filter paper and weighed. The shoots were weighed immediately and then cut into pieces. Both shoots and roots were killed by heating for 20 minutes at 110° C., and then dried for 24 hours at 65°—70° C. At the end of this drying period the dry weights were determined and the material was ground in a waring blender and stored until the next winter months. The phosphorus content was estimated colorimetrically on samples of $\pm$ 100 mg dry weight. The dry material, especially that

of the shoots, being rather heterogeneous, all determinations had to be done in triplicate. In spite of this the determinations had to be repeated three times before reliable values were obtained.

The development of the shoots was chosen as a measure of the growth of the whole plants (ALBERDA 1948). For this purpose the distance between the wooden disc and the top of the youngest visible leaf was measured every 12 or 24 hours at the renewal of the solutions. The average increase of this distance for the 8—12 plants of each set was then taken as a measure of the growth of the plants. When a new leaf appeared, the increase in length of this one was determined henceforth. Table I shows the measurements of the individual plants of the high salt set, used in experiment 6*e* (fig. 13). As could be expected, the course of the growth of a single plant, estimated in this way, was rather irregular. Especially, when we passed on to a new leaf, a sudden fall occurred. If with a number of plants of one set a new leaf happened to appear, this irregularity occurred also in the average value. With the set of table I this was not the case; the course of the average increase in length being therefore very regular. Moreover, the regularity in the results of the experiments was increased by calculating the average growth rate of a number of sets. Of course, the regularity was greatly reduced when the number of plants was smaller (8 instead of 12) and the lapse of time between two determinations longer (24 h. instead of 12 h.). For, the probability that many of the plants produced a new leaf at the same time was increased by the longer time period, the disturbing effect not being mitigated by the greater number of the plants. This was the case in the experiments concerning the relation of growth and uptake. Therefore, in these experiments the number of young leaves which had just appeared was also given. Both data gave an impression of the development of the shoots, a rather crude index of the growth of

TABLE I

The course of the growth of the shoots of nine high salt plants. The increase in length (cm/12 hours) of the youngest visible leaves was determined. The appearance of a new leaf is indicated by *. Compare exp. 6*e* and fig. 13.

Plant number	1 day		2 days		3 days		4 days		5 days		6 days	
1	3.8	2.9	3.6	3.1	3.7	3.8	3.5	2.9	3.2	2.0	2.4	2.4
2	3.1	2.6	2.8	2.8	3.4	2.7	3.4	3.4	3.5	3.0	3.4	2.2
3	3.5	3.4	3.4	2.7	3.5	3.3	4.0	3.7	4.3	2.6	3.8*	3.1
4	2.3	6.9	2.3	2.2	2.5	2.0	2.4	2.5*	2.2	1.3	1.4	0.9
5	1.2	1.5	1.8	1.4	1.1	0.8	1.4	0.8	1.3	0.6	1.3	0.9
6	3.8	4.2*	3.5	2.7	3.9	4.0	4.7	4.4	5.2	4.1	5.2	3.8
7	3.5	3.2	4.0	3.4	3.9	3.1	4.0*	3.1	3.6	2.9	3.6	3.3
8	3.1	2.5	3.4	3.1	3.5	2.8	3.4	2.5	3.5	1.4	2.6	2.6
9	2.9	3.0	2.5	2.3	3.1*	2.0	3.4	2.8	3.2	2.7	3.2	2.8
Average growth rate	3.0	2.8	3.0	2.6	3.2	2.7	3.4	2.9	3.3	2.3	3.0	2.4

the plants, indeed. With these experiments an essential objection to this way of measuring growth came to the fore. The growth measured depends mainly on cell elongation. On the other hand, uptake proved to be connected with synthetic processes. Therefore it is more closely concerned in cytoplasmic growth. It is true that the extension growth depends on meristematic activity but the quantitative relation is rather weak. Hence, the extension growth may continue for some time, the fundamental synthetic processes being nearly stopped. It will be clear, that more exact methods of measuring extension growth (McINTYRE and WILLIAMS 1949), though perhaps giving more regular results, would have failed to overcome this fundamental difficulty. If it would have been possible to measure the rate of protoplasmic growth (e.g. as an increase in dry weight or protein content), no doubt, more striking results would have been obtained regarding the course of the growth and the relation between uptake and growth.

III. EXPERIMENTS

1. THE LOSS OF SALTS BY THE ROOTS INTO THE MEDIUM

Anions are generally assumed to permeate very slowly into the protoplasm, an active absorption being necessary by which anions are transferred from the medium through the membrane into the cytoplasm. The phenomenon of leakage of salts, so often observed in older plants, points however to an easy permeation in the opposite direction. Such a loss also occurred in one of the experiments over the relation of uptake and external concentration. This experiment, together with some experiments in which an attempt was made to obtain the phenomenon once more, will now be described and discussed.

Experiment 1a. A set of low salt plants was used to trace the effect of the external concentration on the uptake. The results of this will be described in section 4. It is important that the maximum absorption amounted to 2·4 mg P_2O_5/2h. On the second day the absorption was rather higher; the maximum rate of uptake was 3·0 mg P_2O_5/2h. It was then attempted to bring the plants rapidly into a high salt condition. For this they were placed for 2½ days on a double-strength Hoagland solution. At the same time the solution was vigorously aerated and the plants were put under a weak illumination in order to reduce the sugar content of the plants as much as possible. Then the effect of the external concentration on the rate of uptake was examined once more. The effect was studied successively of a 25, 50, 75, 100, 150 and 200 % Hoagland solution. The solution of the lowest concentration was taken first. After an absorption period of two hours it was replaced by a solution of a slightly higher concentration etc. The rates of uptake were:

—11·4 —7·0 —0·2 2·2 2·8 2·8 mg P_2O_5/2h.

So a high loss of phosphate at the start of the experiment was apparent. Doubtless this was favoured by the low concentrations. It is striking that, at the end of the experiment the same rate of uptake was attained as was obtained a few days before. The results of the nitrate absorption were:

—10·0 —7·2 —6·0 —7·6 —12·0 —18·0 mg. N_2O_5/2h.

Also in this case a high loss, decreasing during the first periods, but increasing again with the higher concentrations, was obtained. This last result is not so easily understood. At any rate this result shows that there may be a loss of nitrate and a normal uptake of phosphate at the same time. The next day the absorption of phosphate was quite normal again. With rising concentration, the rates of uptake were:

1·0 2·1 2·5 3·0 2·9 2·8 mg P_2O_5/2h.

The uptake at the highest concentrations again showed the same maximum rate as it did at the start of the experiment.

Experiment 1b. Just as for the first experiment a low salt set (cultivated August—September 1950) was used. It was put immediately on a Hoagland solution of double-strength to bring it into a high salt condition. Moreover, the light intensity was reduced after two days. On the fourth day the set was placed on a half-strength Hoagland solution. The solution was renewed every two hours. The course of the uptake was:

—0·78 0·00 0·64 0·77 0·93 1·14 mg P_2O_5/2h.

Then the plants were placed in darkness for two days. A minus-nitrogen nutrient solution (200 %) was used. After this treatment the uptake on a 50 % Hoagland solution amounted to 0·32 and 0·21 mg P_2O_5/2h. There was no loss of phosphate. The treatment was continued immediately for another two days. The rate of uptake was then: 0·00 and 0·40 mg P_2O_5/2hours.

After this the set was placed on a double-strength complete Hoagland solution again. The next day the uptake was determined again on a half-strength solution:

—1·57 —0·49 0·03 0·07 mg P_2O_5/2hours.

So there was a loss of phosphate after a dark treatment, the plants being placed on a nutrient solution of high concentration. A high nitrate concentration proved to favour particularly the loss of phosphate.

Experiment 1c. For this experiment a set of low salt plants was used again (grown August—September 1950). As a result of the unfavourable circumstances the vegetative growth was greatly checked, so that the plants though being 7—8 weeks old, were but little more developed than were the previous sets. On the other hand

these plants showed an incipient flowering. In order to bring the plants into a high salt condition they were placed for two days on a full-strength Hoagland solution. The plants remained in the light throughout the experiment. The uptake was studied on the same day on two different concentrations. On a full-strength Hoagland solution the course of uptake during three successive absorption periods was:

—0·46 0·26 0·42 mg P_2O_5/2h.

Immediately after this on a weaker solution viz. 25 % Hoagland solution:

—0·36 0·04 0·12 mg P_2O_5/2h.

On lowering the concentration a loss was obtained which soon changed into a normal active uptake again.

Then the pretreatment was continued; after this the uptake resp. loss of phosphate was again estimated on three different concentrations during nine successive absorption periods. The results were:

On the 200 % Hoagland solution:

—0·40 —0·20 —0·18 mg P_2O_5/2h.

Then on the 100 % Hoagland solution:

—0·22 0·10 0·08 mg P_2O_5/2h.

Finally on the 25 % Hoagland solution:

—0·09 0·07 0·07 mg P_2O_5/2h.

The increase of the uptake was less pronounced in this case as a result of the highly diminished absorption activity. Because of this the differences at the transition to a solution of lower concentration were less striking.

Experiment 1d. In this experiment a high salt set was used, grown under artificial illumination (November 1950). The plants were three weeks old and displayed a vigorous vegetative growth. Because of this they were not in an extremely high salt condition. Therefore the set was put on a complete Hoagland solution for four days and then in darkness on a solution of double-strength for two days. No loss was obtained either on a double-strength Hoagland solution or on a full-strength solution or a half-strength one. The pretreatment was continued for a week on a double-strength Hoagland solution in darkness. After this the course of the uptake on a full-strength solution was:

? —1·44 —0·88 —0·48 0·00 0·16 mg P_2O_5/2h.

It can be concluded that only by a dark treatment of long duration could a loss be obtained with young, vigorously developing material.

Discussion

It has been known for a long time that roots may show a loss of substances. Originally interest was directed towards the acids and organic compounds (dissolving capacity; interaction problems). Later on, inorganic substances were also found to be excreted. It remained uncertain for a long time if this loss was based on an injurious effect, a dying off of the rootcells, or on a normal physiological function. That the latter probability may apply was proved by an extensive investigation of ACHROMEIKO (1936).

Many investigators have pointed out the importance of the phase of development to the rate of uptake and loss. This conclusion has been mainly based on analysis of material at various moments during the development. Mostly it is only the aerial parts that have been harvested and analysed. The amount of inorganic substances was often found to diminish during the flowering stage. There must have been a loss of salts from the shoots to the roots. Though a proof has not been given of the hypothesis that ions are lost by the roots, this is very probable. The salt content of the roots is tending to decrease rather than to increase during this stage (ACHROMEIKO).

It is obvious that the occurrence of leakage depends on the external conditions e.g. the availability of nutrients. Besides this however, the type of the plant is important. Loss of substances is generally found with plant species showing a marked difference between the vegetative stage and the flowering one (MAXIMOV 1938). Potassium is always lost very easily but a loss of phosphate is often obtained too (HORNBERGER 1882; BURD 1919; WILFARTH, RÖMER und WIMMER 1906; ACHROMEIKO 1936; DELEANO und ANDREËSCO 1932; ASANA 1949; KNOWLES and WATKIN 1931; VAN DER PAAUW 1948; WILLIAMS 1948).

In physiological experiments a loss by the roots was also met with. Thus, HUMPHRIES (1950, '51) found with excised root-systems a loss of phosphorus, nitrogen and potassium. Particularly potassium is readily given off e.g. as a result of a dark treatment (LUTTKUS und BÖTTICHER 1939; VAN ANDEL, ARISZ and HELDER 1950).

In accordance with this evidence from the literature it was found that with plants already in the flowering stage, a loss of phosphate appeared easily. Younger vigorously developing material being used, a check of the development e.g. by a dark treatment was necessary. A second condition was a pretreatment with a nutrient solution of high concentration. The effect of the nitrate concentration was remarkable. The meaning of this is not yet quite clear. Achromeiko pointed out the fact that an excretion by the roots during the flowering stage mainly occurred with plants rich in proteins. He assumed a relationship between protein metabolism and loss of minerals. This enhanced the impression of the plants having to be in a condition comparable with that of plants in the flowering stage. Then dissimilation is dominant, resulting in an increase of the concentration of non-metabolized substances. As soon as the external concentration is lowered a certain amount of salt will be given off until a new equilibrium has been established.

Where the phosphate lost came from was not traced. From the literature mentioned above it can be concluded that with plants in the flowering stage the shoot particularly looses the substances. This concerns however a loss during a prolonged period. LUTTKUS und BÖTTICHER showed that the potassium excreted by the roots in darkness, was mainly derived from the shoot. However, potassium is generally assumed to be easily transported. Anions will be transferred much more slowly. Hence, the phosphate given off in these short experiments, may be supposed to come nearly exclusively from the roots. By this it is not yet decided whether the ions come from the symplasm or from the vacuoles.

The exosmosis of substances by cells or tissues may be the result of an injurious effect e.g. by a sudden change of temperature of the medium; by using unbalanced solutions, narcotics or toxic substances. By this the semipermeability of the cytoplasm is lost most often, so that substances of the vacuoles may permeate outwards e.g. the anthocyanin of the beetroot. It is not very likely that the loss by the roots of the experiment mentioned here, would be the result of damage, for the rate of loss of phosphate decreased rapidly, and changed into an uptake that could be as high as the uptake was before the plants had been brought into a condition favourable to loss. Moreover a loss of nitrate was found to occur, the phosphate being absorbed actively at the same time. Hence it may be concluded that the loss of salts from the symplasm is mainly or perhaps exclusively concerned. This might mean that the symplasm is highly permeable to salts. ARISZ (1945) and WIERSUM (1947) arrived at the same conclusion. For a complete exposition of the subject and a discussion of the literature these authors may be referred to. With the exception of the data about the loss of ions they point to the experiments of RUFZ DE LAVISON (1910); the promoting effect of the transpiration stream on the absorption (FREELAND 1937; SCHMIDT 1936); the exchange phenomena as they have been especially studied with radioactive substances (OVERSTREET and BROYER 1940; BROOKS and BROOKS 1941) The resistance so often found in experiments on permeability must be situated mainly in the tonoplast. Meanwhile, ROBERTSON (1950, 1951) also cautioned against an overestimation of the importance of an outer membrane of the cytoplasm. He points to the possibility that a slow diffusion rate of anions may also depend on the presence of large indiffusible organic anions in the cytoplasm. He puts greater value on explanations based on the Donnan-theory. The picture of the process of loss of ions just mentioned, is in accordance with this. The plants being in a normal active condition, the free anion content of the cytoplasm will be low (ARISZ 1945). Only at special conditions, when metabolic activity is low, can the concentration increase and an obvious loss may occur, the external concentration being considerably lowered. Especially the fact that the leakage was dependent on the external concentration made it seem likely to be a passive process.

Attention should be drawn to one difficulty in this concept. One

condition for the occurrence of a Donnan equilibrium between tissue and medium is that the cytoplasm may be considered a gigantic, immobile and polyvalent colloid ion (FREY WYSSLING 1948). In such a cytoplasm, transfer of anions can be expected to be highly impeded, and BRIGGS and ROBERTSON (1948) found diffusion of anions in tissue discs to be very slow indeed. The rate of loss found in these experiments, seems to be in conflict with these findings. However, it is uncertain whether the salts were lost as molecules or as ions. Moreover, it is unknown where the salts came from, so that it is impossible to decide if there has been a transport over a long distance. Compared with the absolute amount of phosphate in the roots (at a rough estimation 20—30 mg P_2O_5) the amounts given off were not high. But to this amount the phosphate, not available or not easily available for loss also belongs. It is impossible therefore, to get an impression of the quantitative meaning of the loss found. No more do the experiments of WIERSUM (1947) give clear results, the data concerning the precise anion permeability of the root tissue being far from complete. In the opinion of the author, they point to a low passive permeability; the phosphate and nitrate transfer obtained can be considered as a normal active secretion into the xylem vessels. On the other hand experiments over the exchange with isotopes point to a rather rapid transfer possibility. Therefore principles other than passive diffusion may play a part e.g. surface migration (BROOKS and BROOKS 1941; JENNY and OVERSTREET 1939). To what extent such phenomena of accelerated diffusion are important, is difficult to say. It may be that it has a meaning for the transport in the walls of the cells of the cortex (ROUSCHAL und STRUGGER 1940), by which the whole of the cortex cells is in direct contact, so to speak, with the medium (PREVOT and STEWARD 1936; WIERSUM 1947). The surface of the cytoplasm now being permeable to anions, the ion content of the symplasm can be brought rapidly into equilibrium with the external concentration.

The leakage is generally assumed to be a passive process. In agreement with this assumption it has been attempted up to now to explain the processes with the aid of physico-chemical principles such as diffusion or accelerated diffusion. This does not exclude other transport principles more closely related to metabolism. Thus, protoplasmic streaming will be of direct importance to the loss of ions, because of its levelling concentration gradients arisen from that loss. The connection between absorption and metabolism will come up for discussion in the next sections. To what extent the leakage has to be considered in an analogous way will be discussed in details in the general discussion.

2. THE INFLUENCE OF PHLORIDZIN ON THE UPTAKE

The view that metabolism, particularly of respiration, is of importance to the salt absorption is nowadays generally accepted. The most important facts that have led to this view are: the capacity to accumulate ions against a large concentration gradient; the necessity of a good oxygen supply; the high dependence on temperature. In these

experiments also the effect of the air supply on uptake was clearly demonstrated in those cases where some stagnation of the aeration occurred. Even in experiments with absorption periods of only two hours, the uptake of phosphate decreased immediately and could sometimes even stop.

In particular, the investigations with inhibitors (LUNDEGÅRDH, MACHLIS, ROBERTSON, ARISZ) have demonstrated the connection between ion absorption and metabolism very clearly. On the grounds of such investigations LUNDEGÅRDH holds the cytochrome-oxydase system responsible for the absorption mechanism. Robertson and collaborators have been able to corroborate this view with discs of storage tissue and pieces of roots, as also has ARISZ with *Drosera* tentacles and leaf strips of *Vallisneria.*

It was quite natural that it was asked, to what extent the phosphorylation processes, being so closely coupled to the dissimilatory processes, have significance for the uptake too (HOAGLAND 1948; NANCE 1949). ROBERTSON, WILKINS and WEEKS (1951) therefore traced the effect of 2,4-dinitrophenol on the active salt absorption. As a matter of fact, they obtained an obvious inhibition. However, they point out the fact that the inhibition may be due to some injurious effect on protoplasmic structures essential to the absorption mechanism.

It is clear that phosphorylation processes may have a binary significance on the uptake of phosphate, phosphorus being used up in these processes too. That is why the influence of inhibition of these processes on the uptake of phosphate was studied. As an inhibitor, phloridzin was used, as it is generally considered to be a harmless substance. Only weak concentrations were used. The experiments were performed in the same way as was described in the previous section. A complete, full-strength Hoagland solution (p_H: 6·4—6·5) served as a nutrient solution. Phloridzin was added up to concentrations of 10^{-4}—10^{-3} molarity. The substance is poorly soluble at room temperature. By heating up to 60° C. this trouble can easily be overcome. It is of primary importance to trace the influence on the uptake of other anions along with the influence on the phosphate absorption. Therefore, in some experiments both the uptake of phosphate and of nitrate were determined. The presence of phloridzin gave some trouble because of its disturbing effect on the phenoldisulphonic acid method. Therefore, a nitrogen determination by the micro-Kjeldahl method was occasionally made. The precision of the determinations of the nitrate uptake was not very high.

This examination had to be cut short. For that reason, results and conclusions are highly preliminary. However, they are in full agreement with the results obtained by ARISZ (1952) with *Drosera* and *Vallisneria.*

Experiment 2a. In this experiment two sets were used, each consisting of twelve, three week old plants. To be sure of a sufficient rate of uptake, absorption periods of three hours were taken. During

the first day the plants were intensively illuminated. There were four absorption periods. During the second and the fourth period, phloridzin was added to the solution.

The first set was placed first on a concentration of 10^{-3} mol. phloridzin and then on a concentration of $3 \cdot 2 \cdot 10^{-4}$ mol. The rates of uptake were:

Phloridzin concentration:

0	10^{-3}	0	$3 \cdot 2 \cdot 10^{-4}$ mol.

Uptake:

1·73	*1·42*	1·10	*1·08*	mg P_2O_5/3h.
18·2	?	20·1	?	mg N_2O_5/3h.

There was an obvious inhibition of the uptake of phosphate at the higher phloridzin concentration. The inhibition was continued during the third absorption period when phloridzin was no longer applied. Hence there was an after-effect. The low concentration did not show any influence.

The nitrate determinations of this experiment were made colorimetrically. Phloridzin proved to interfere. Hence, the nitrate uptake could not be determined during the second and fourth absorption period. There was no inhibition of the nitrate uptake during the third period, as was the case with the uptake of phosphate.

The second set was placed first on a low concentration of phloridzin (10^{-4} mol.) then on a rather higher one ($3 \cdot 2 \cdot 10^{-4}$ mol.). The course of uptake was:

Phloridzin concentration:

0	10^{-4}	0	$3 \cdot 2 \cdot 10^{-4}$ mol.

Uptake:

1·32	*1·32*	1·36	*1·31*	mg P_2O_5/3h.
18·2	*19·2*	22·3	?	mg N_2O_5/3h.

There was no question of any inhibition either of the phosphate uptake or of the nitrate uptake.

Summarizing, it can be stated that phloridzin could have an inhibiting influence on the phosphate absorption. Whether the nitrate absorption can be inhibited too, could not be settled. In any case the nitrate absorption turned out to be less sensitive to the addition of phloridzin.

Both sets were subsequently placed in darkness. The next day the inhibiting effect of phloridzin was investigated again. At the same time the influence of glucose addition (2 gm per 300 cm^3 solution) was examined. The first set was successively put on a Hoagland solution, a solution with phloridzin ($3 \cdot 2 \cdot 10^{-4}$ mol.), a solution with both phloridzin and glucose, and a solution only with glucose. The rates of uptake were:

1·17	0·77	0·88	1·13	mg P_2O_5/3h.

As a result of the dark treatment the level of the uptake activity was lower than it was the day before. Moreover, there was an in-

hibition of the uptake at a phloridzin concentration of $3 \cdot 2 \cdot 10^{-4}$ mol. By adding glucose the uptake was but little increased. The phloridzin being removed, the uptake rose immediately however and there was no after-effect.

The second set was treated in the same way only the phloridzin concentration was slightly higher viz. $5 \cdot 6 \cdot 10^{-4}$ mol. The course of the uptake was:

1·04 0·42 0·73 0·99 mg P_2O_5/3h.

The course was comparable with that of the first set. The inhibition was much stronger and so was the favourable influence of the glucose, even in the presence of phloridzin.

In all cases in which phloridzin had been added, the solutions were faintly yellow in colour after having been in contact with the roots. The colour intensity was proportional to the phloridzin concentration used. With the lower concentration (10^{-4} mol.) the colour could hardly be distinguished. If the solutions used were stored, the colour turned deeper. Contrary to this, solutions with phloridzin, not having been in contact with the roots, were only after days coloured very faintly. Also the solution without phloridzin, used after a period in which phloridzin had been added, were obviously, though more faintly coloured. This could not be a consequence of some residue of the solution used before, for the roots were rinsed thoroughly when changing the media. STREET and LOWE (1950) impute this colouring to some impurity of commercial phloridzin: in their opinion it cannot be due to hydrolysis of phloridzin. At any rate, both the velocity at which colouring appeared in these experiments and the fact that an obvious after-effect sometimes could be seen, do point in that direction. Phloridzin has a large molecule and permeates passively with difficulty, as does raffinose. But the after-effect of the inhibition and the colouring of the solution without phloridzin used afterwards, pointed to a permeation of the phloridzin.

The same sets were used in the next experiment, the plants being five weeks old. Phloridzin concentrations of 10^{-4}—10^{-3} mol. were successively added. The maximum inhibition amounted to 41 %. This was already attained at $4 \cdot 6.10^{-4}$ mol. There was an obvious after-effect. On the second day there was even some stimulation of the uptake at a low concentration. Such a stimulation also occurred after the addition of glucose. If besides, phloridzin was added to this solution with glucose no alteration of the uptake was observed.

Experiment 2b. From the previous experiment it appeared that there can be an after-effect of the phloridzin inhibition. Because of this it is impossible to trace the influence of the phloridzin concentration by putting the plants successively on solutions with varying amounts of phloridzin. Therefore on five consecutive days of this experiment the uptake was determined during five absorption periods every day, on a complete Hoagland solution, phloridzin being added during the third absorption period. In this way the effect of four

different concentrations of phloridzin was studied. Two sets of twelve plants were used, a high salt set and a low salt one. They had been grown under unfavourable circumstances (August—September 1951). That is why they were put into the room of constant temperature five days before the experiment and cultivated under artificial light. They must have been in a high sugar condition. The results are summarized in table II. With 10^{-4} and $2 \cdot 1.10^{-4}$ mol. there was a

TABLE II

The influence of various phloridzin concentrations on the uptake of phosphate. The phloridzine was applied during the third absorption period of each day at the concentration indicated.

Phloridzin concentration	1	2	3	4	5	
						High salt
10^{-4}	1.44	1.49	*1.70*	1.28	1.26	mg P_2O_5/2h
2.1×10^{-4}	1.36	1.32	*1.60*	1.19	1.17	,,
4.5×10^{-4}	1.39	1.45	*0.87*	0.91	0.81	,,
10^{-3}	1.17	1.49	*1.04*	1.10	1.14	,,
10^{-4}	1.05	0.95	*1.30*	1.12	1.08	,,
						Low salt
10^{-4}	0.04	0.43	*0.47*	0.36	0.43	mg P_2O_5/2h
2.1×10^{-4}	0.70	0.75	*1.02*	0.66	0.74	,,
4.5×10^{-4}	1.07	0.85	*0.44*	0.34	0.31	,,
10^{-3}	0.84	0.94	*0.51*	0.68	0.71	,,
10^{-4}	0.72	0.72	*0.95*	0.84	0.85	,,

stimulating effect on the phosphate uptake; with $4 \cdot 5.10^{-4}$ and 10^{-3} mol. an inhibition. Again there was an after-effect of the phloridzin treatment. It was striking that this after-effect, on the first two days consisted of an inhibition, though a stimulation was found in presence of phloridzin. On the last day, as a stimulation was obtained too, the decrease afterwards was less pronounced. Hence in spite of this decrease the rate of uptake remained a little higher than it was during the first two absorption periods. The absorption level on this day however, was somewhat lower than it was during the previous days.

Experiment 2c. The last experiment, in which an inhibition was obtained, was performed in October 1951. It was necessary to grow the material during the last week in the room of constant temperature under artificial light. The uptake during five periods of two hours was determined. During the third period phloridzin was added in a concentration of $5 \cdot 10^{-4}$ mol. The uptake of nitrate was also determined by analysing samples with the aid of the micro-Kjeldahl method. The results were:

Set I	0·80	0·80	*0·64*	0·80	0·83	mg P_2O_5/2h.
Set II	0·50	0·51	*0·33*	0·53	0·66	mg P_2O_5/2h.
Set I	92·9	92·3	*89·4*	91·6	91·8	mg N_2O_5/2h.
Set II	94·7	94·0	*90·8*	104·0	104·0	mg N_2O_5/2h.

No doubt, the nitrogen determination supplied absolute values too high for the nitrate uptake. These figures have therefore only a relative value. At any rate, both the phosphate and the nitrate uptake were inhibited. The inhibition of the phosphate uptake amounted to 20— resp. 37 %; the values for nitrate were 3— resp. ± 5 %. There was no clear after-effect.

Discussion

In an occasional supplementary experiment no inhibition was found. The material of this experiment was in bad condition (grown October 1951) and showed only a slight absorption. It was remarkable that the solutions were not coloured in these cases. Thus, there is a relation between inhibition and after-effect on the one hand and the colouring of the solutions on the other hand. This needs further examination. The importance of the condition of the material may be derived from the results concerning the influence of light and darkness and of the glucose supply on the action of phloridzin. This last might point to some competition between sugar and phloridzin, as was assumed by STREET and LOWE (1950), as to the phosphorylation processes, enabling the sugar absorption to take place. This assumption too requires further investigation.

This preliminary study has clearly shown the possibility of checking the uptake of phosphate, while at the same time perhaps the uptake of nitrate is checked to a far lesser degree. This last does not necessarily imply that phosphorylation processes are without importance to the nitrate absorption. It only makes it seem very likely that the phosphate uptake is dependent on this process to a much higher degree. This is quite understandable if the phosphate uptake is assumed to be determined by the measure in which phosphate is used up in these phosphorylation processes. This assumption is in accordance with that which is known about phloridzin action. Though there is no agreement as to the way in which phloridzin checks these phosphorylations, it is certain that the direct or indirect result is a blocking of the formation of hexose-phosphate (SHAPIRO 1947). Besides this, investigations with radioactive isotopes have shown that instable phosphate esters (gel-P: Green 1951), adenosine tri-phosphate and hexose-phosphate belong to the first and very rapidly formed products of the phosphate metabolism (HAUROWITZ 1948). It seems legitimate to conclude, that the formation of an organic phosphorus compound constitutes an essential part of the phosphate absorption process. Moreover, it must be primary in every form of phosphate uptake, since inhibition by phloridzin occurs with low salt as well as with high salt material. This view will be further dealt with particularly in the section on the relation between uptake and external concentration.

3. THE IMPORTANCE OF THE SUGAR SUPPLY FOR THE UPTAKE OF PHOSPHATE

If the uptake of phosphate is so intimately connected with metabo-

lism (respiration, phosphorylation), the sugar condition of the plants can be expected to be of primary importance. The significance of the salt condition for the uptake is well-known (HOAGLAND and BROYER 1936). Many experiments, for that reason, are performed with high salt as well as low salt material. Now the roots of both types of plants differ in sugar content too. As could be expected, ALBERDA (1948) found roots of low salt plants to have a higher sugar store than have high salt plants. Therefore, one can wonder if the differences between the uptake, in particular between the uptake of phosphate of low salt and high salt plants should not be ascribed to the sugar condition rather than to the salt condition. In this relation PHILLIS and MASON's finding (1940 *a*, *b*), that with cotton plants the uptake is to a high extent dependent on the supply of substances from the shoot, is important. Ringing the plants depressed the uptake of bromide by the roots within a few hours. When the plants had been amply illuminated, the effect was much less.

Alberda investigated the influence of light and darkness on the phosphate uptake of high salt plants. In darkness the rate of uptake decreased rapidly. However, the growth of the shoots showed a similar course. Since the plants were in a high salt condition, ALBERDA assumed the growth of the shoot to have a determining influence on the uptake by the root. As a result of the diminished utilization, a part of the phosphate supplied by the roots, was carried back to the roots, which had a damping effect on the uptake from the external solution. So in the first place the salt condition was held responsible for the course of the uptake found.

In order to trace the importance of the sugar supply for this course of the uptake a few experiments were performed, in which the plants were brought into a carbon dioxide-free atmosphere. As will appear, results could be obtained virtually equal to those obtained by a dark treatment. Besides, further inquiries into the influence of glucose addition to the nutrient solution were made. As was found already by VAN ANDEL, ARISZ and HELDER (1950), the phosphate absorption is increased by sugar supply even in the light. These experiments were only carried out with high salt plants. They were continued and extended by the author. In addition to high salt, low salt material was also examined. Moreover, an attempt was made to trace the extent to which the growth of the shoot may have an influence on the uptake, at the same time.

Experiment 3a. To trace the influence of the withdrawal of carbon dioxide on the phosphate uptake, two sets of twelve high salt plants, six weeks old, were used (grown June—July 1948). Use was made of the complete arrangement, shown in fig. 1: A. On the first day the uptake was determined with the case opened. After this the case was closed. Carbon dioxide-free air was led through for four days. Finally normal air was used for six days. On the last day the case was opened again. Figure 2 represents the average result of the two sets. It clearly shows that the uptake immediately fell on carbon dioxide deficiency.

The course can be completely compared with the behaviour of the plants coming into the darkness (fig. 12, 13). The increase of the uptake, the plants being in normal air again, was slight but steady,

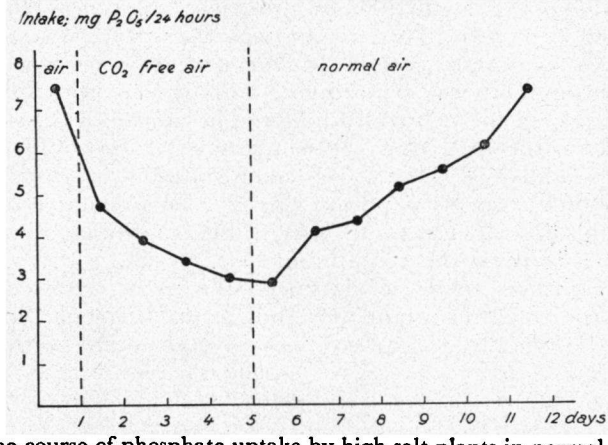

Fig. 2. The course of phosphate uptake by high salt plants in normal and carbon dioxide free air. The plants were illuminated continuously.

so that in the end the original level was nearly obtained. It is possible that the isolation of the shoots in a confined space constituted less favourable circumstances.

Experiment 3b. A corresponding experiment was also made with low salt plants. The plants were in the closed case throughout the experiment. With two sets, normal air was led through during the first two days and then carbon dioxide-free air during the last two days. A third set was placed immediately in carbon dioxide-free air and afterwards into normal air. The results are represented by table III.

TABLE III

The influence of the withdrawal of carbon dioxide on the course of the uptake of phosphate by low salt plants (exp. 3*b*)

	1 day	2 days	3 days	4 days	
	+ CO_2		— CO_2		
Set I	10.5	9.8	9.0	8.4	mg P_2O_5/24h
Set II	10.6	10.4	10.4	6.2	,,
	— CO_2		+ CO_2		
Set III	13.0	7.7	5.8	6.7	mg P_2O_5/24h

Low salt material has a higher sugar content and as a matter of fact only a slight effect from the withdrawal of carbon dioxide was obtained with set I. The other sets showed an obvious decrease as did the high salt sets. This gradual difference between low salt and high salt material has also been found in the experiments concerning the influence of light and darkness (fig. 10).

Experiment 3c. A low salt set was grown under artificial light. The plants were then in a high sugar condition. With these plants the effect of sugar supply was traced. The set was placed on the root chamber (fig. 1:C) with 400 cm^3 nutrient solution. This solution was changed every two hours. In this way the uptake was determined during seven two-hours periods on one day. The set was placed in darkness throughout the experiment. This is why the sugar supply could be expected to decline gradually. The course of the uptake was determined on the first, third, fifth and seventh day. On these days glucose was added (2 gm per 400 cm^3 solution) during the third, fourth and fifth absorption period (fig. 3). To prevent the plants as far as possible from getting into a high salt condition, they were put on tap water during the remaining days.

In spite of their being in darkness, the plants showed a larger uptake on the third day than they did on the first one. Such an increase of the phosphate uptake was always found with low salt

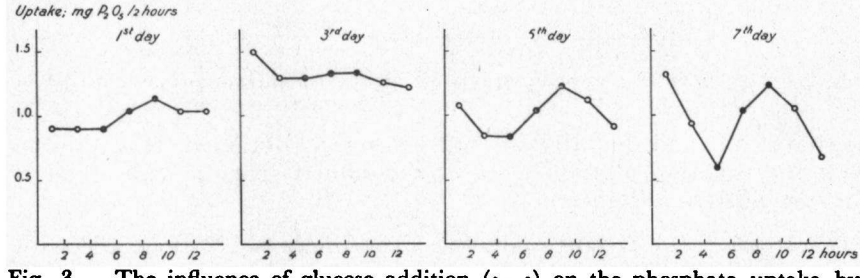

Fig. 3. The influence of glucose addition (•—•) on the phosphate uptake by low salt plants in darkness.

material, as will appear from the next section. It depends on the lack of salts, in particular, on phosphate deficiency, by which the growth and the absorption activity is impeded. The increase occurred in the dark in this experiment. This means that the sugar supply was not yet a limiting factor. Accordingly it is understandable that addition of glucose as yet had no effect. Moreover, it is remarkable, that the uptake showed a decreasing tendency at the start of the third, fifth and seventh day. It is possible that this higher uptake during the first absorption period depended on the plants having been on tap water continuously for about 38 hours. The influence of glucose administration was becoming more pronounced during the course of the experiment. Whereas hardly any influence could be observed on the first and third days, there was a more obvious effect on the fifth day. And on the last day as the plants had been in darkness for a week, the effect was very striking.

Experiment 3d. A high salt set was used, grown under the same circumstances as the low salt set of the previous experiment. As will appear, this set had at its disposal a sufficient amount of sugar. With respect to methods this experiment was like the previous one. On the first day it was traced to see whether the administration of various

amounts of glucose brought about an enhancement of the uptake. Successively 0·5, 2 and 4 gm glucose were added to 400 cm³ nutrient solution. None of the concentrations used had any effect (fig. 4).

The next day the shoots were excised at the end of the first absorption period. If a part of the amount of phosphate supplied by the roots was transported back from the shoots, owing to the dark treatment, an enhancement of the uptake from the medium could be expected to occur as soon as the shoots were removed. In fact, the uptake continued undisturbed at the same rate during the whole day. For that reason, an important loss from the shoots did not occur under the given conditions. Some exudate appeared at the stumps,

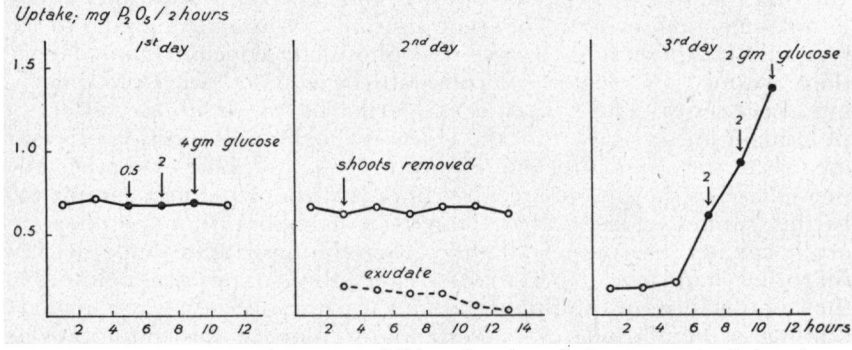

Fig. 4. The influence of glucose addition (•—•) and the removal of the shoots on the phosphate uptake by high salt plants.

that was blotted with filterpaper. The amount of phosphate present in this, was determined. The volume of the exudate decreased rapidly during the course of the day. At the end hardly any exudate was to be seen. As figure 4 indicates, the total amount of phosphate decreased too. (The method of estimation was rather a crude one).

On the third day the rate of uptake had become much lower. At this time the addition of 2 gm glucose enhanced the uptake immediately. It was very striking here that the enhancement continued to increase, so that finally the absorption rate of the intact plants on the first day was surpassed. The next day the uptake was low again (not represented by fig. 4). Addition of glucose had but slight effect.

Experiment 3e. The previous experiment was repeated in a somewhat different way with an older and more developed high salt set. After the uptake of the intact plant had been determined, the three uppermost, rapidly growing leaves of all eight plants were drawn loose. There remained on every plant three full-grown leaves. During the three following periods no marked influence was to be seen. At the end of the fourth period shoots were entirely excised as was done in the previous experiment. In this case the uptake fell immediately. This fall could be changed into an increase by adding glucose during the last absorption period. The exact course of uptake was:

6·0 6·0 6·2 5·6 2·2 3·4 mg P_2O_5/2h.

This result supports the assumption that the sugar supply from the shoot has a strong influence on the phosphate uptake from the medium.

Discussion

All experiments mentioned, confirmed the supposition that the sugar supply is very important for the phosphate uptake. HUMPHRIES (1950, 1951) found with excised root systems of barley plants that the uptake of nitrate and phosphate was enhanced by the administration of sugar (BROYER 1951). In particular, the phosphate uptake was sensitive.

STREET and LOWE (1950) studied the inverse relationship. They found sugar absorption by root cultures of tomato plants to be favoured by increasing the external phosphate concentration. Hence they assumed the sugar absorption to be coupled with phosphorylation processes. This idea was supported by the inhibitory effect of phloridzin on the growth of the cultures. Such an absorption of sugar means at the same time an absorption of phosphate. Whether the first phase of the phosphate absorption can be explained completely by this, cannot yet be decided, though it renders the intimate connection of phosphate absorption and the sugar relations understandable. On the other hand the experiments of the previous section pointed to the significance of phosphorylation processes, so that a chemical binding of the phosphate as an essential step in the absorption process became likely.

In any case, the stimulating influence of sugar addition also on the uptake of other ions, indicates that sugar influences the absorption process in a more general way as well, e.g. by enhancing the growth. PREVOT and STEWARD (1936) found the uptake of bromide by excised high salt roots to be enhanced to such a degree by 2 % glucose, that even the absorption level of low salt roots was attained. They hold the limited growth of excised high salt — low sugar roots responsible for the low absorption velocity. With intact plants growth of the roots is possible as a result of sugar supply from the shoots. At the same time a transport of salts into the shoots is possible.

The effect of the growth of the shoots could not be demonstrated. It will be clear that the dominating significance of the sugar supply for phosphate uptake does not mean that the growth of the root and shoot are of no importance to the uptake. On the contrary, the influence of sugar may depend entirely or partly on its effect on the growth. Further evidence is necessary however. This will be produced in the next section.

4. THE RELATION BETWEEN THE RATE OF PHOSPHATE UPTAKE AND THE EXTERNAL CONCENTRATION

Many investigators have determined the relationship between external concentration and the absorption velocity. It was generally found that this relationship could be represented by an absorption curve of the shape of the adsorption-isotherm. For this reason, van

den Honert assumed the first stage of the absorption process to consist of an adsorption process (1933, 1936).

ALBERDA (1948) studied this relation with high salt and low salt plants. A remarkable difference between these two types of material was that high salt plants attained their maximum rate of absorption at a lower concentration than did the low salt plants. To be able to explain this difference, Alberda assumed that, by raising the external concentration, the uptake became so large, that the transfer of phosphate into the shoots surpassed the fixation by growth. The surplus will be returned to the roots, having a damping effect on the uptake from the medium (MASON and MASKEL 1931; VAN DE SANDE BAKHUYZEN 1937).

Seeing that low salt and high salt plants also differ in sugar content, these experiments were repeated. At the same time the influence of addition of sugar on this relation and on the saturation phenomenon was traced. All experiments were carried out using the root chamber (fig. 1: C). This rendered a rapid replacement of the solution possible. The sets of plants were placed on 400 or 350 cm^3 Hoagland solution of various concentrations (6·25—200 %). The p_H of the solutions was adjusted to 6·2—6·4 with sulphuric acid. To trace the effect of sugar, 2 gm glucose was added to the solutions. As a rule six different solutions, of increasing concentration, were successively applied. No essential differences were found if the solutions were applied in an inverse sequence.

The absorption periods being short, the amount of phosphate taken up was but slight. With low concentrations especially, the error of the determination of the phosphate uptake can become significant. It amounted to 0·12 mg P_2O_5/2h. at a maximum, as far as it depended on the precision of the phosphate determinations of the solutions used. At the lower concentrations it was somewhat less.

A regular periodicity of the phosphate uptake did not occur during the course of the day, but a more or less sudden change could occur. This rendered a great number of experiments necessary. In spite of this it is not possible to illustrate the regularities in the relation between concentration and uptake and the effect of glucose supplied by a single experiment.

To judge the relationship existing between the rate of uptake and the external concentration, the rates of uptake with the corresponding concentrations had to be determined. As a result of the uptake the concentration alters during the absorption period together with the rate of uptake. The velocity, obtained by analysing the solution at the start and at the end of an absorption period gives, as a matter of fact, only an average value. Now the question arises, which external concentration does this average rate of uptake belong to. As a first approximation the arithmetic mean of initial and final concentration may be used. To judge whether the points, plotted in this way, fit into an adsorption-isotherm, we can make use of the empirical formula of Freundlich:

$$u = k c^{\frac{1}{n}}$$

Whether the relation between the rate of uptake u and the external concentration c may be represented by Freundlich's equation can easily be seen by plotting both data logarithmically. If they satisfy this equation, a straight line will be found, for:

$$\log u = \frac{1}{n} \log c + \log k$$

The curve of the closest fit may be calculated and hence the coefficient k and the exponent $1/n$ are known and the absorption curve can be calculated and drawn.

As will appear from the experiments, the relation can certainly be represented by Freundlich's equation, at least within a certain range of concentrations. However this means at the same time that the choice of the arithmetic mean of initial and final concentration is, theoretically, not a correct one. It is possible to make an estimation of the error.

As a result of the uptake u, the concentration c of the solution falls. The intensity of this alteration depends on the volume V. The amount of phosphate absorbed during a time interval dt amounts to $u.dt$ or $k.c^{1/n}\ dt$. The resulting change of the concentration c_t can be represented by:

$$dc_t = -\frac{k.c_t^{\frac{1}{n}}}{V} \cdot dt$$

Hence:

$$dt = -\frac{V}{k} \cdot \frac{1}{c_t^{\frac{1}{n}}} \cdot d\,c_t$$

Integration gives:

$$t = -\frac{V}{k} \int \frac{1}{c_t^{\frac{1}{n}}} \cdot d\,c_t = -\frac{V}{k} \left(\frac{n}{n-1} \cdot c_t^{\frac{n-1}{n}} + C \right)$$

Assuming the initial concentration at $t = o$ to be c_o, the integration constant C follows from:

$$C = -\frac{n}{n-1} c_o^{\frac{n-1}{n}}$$

The relation between t and c now becomes:

$$t = \frac{V}{k} \cdot \frac{n}{n-1} \cdot \left(c_o^{\frac{n-1}{n}} - c_t^{\frac{n-1}{n}} \right)$$

The average value of the absorption velocity belongs to some concentration c, lying between c_o and c_t. The rate of uptake at this concentration is $k.c^{1/n}$. If this uptake takes place at this constant rate during the time t, a concentration decrease is obtained equal to

c_o—c_t, so that the formula becomes:

$$\frac{k\, c^{\frac{1}{n}}\, t}{V} = c_o - c_t$$

Hence:

$$c^{\frac{1}{n}} = \frac{c_o - c_t}{\frac{n}{n-1}\left(c_o^{\frac{n-1}{n}} - c_t^{\frac{n-1}{n}}\right)}$$

Thus it is possible to calculate the correct concentration that the average rate of uptake obtained belongs to. Meanwhile, it appeared that there was only a slight difference between this correct value of the concentration and the mean value of initial and final concentration. This was mainly due to the short absorption period, as a result of which only a slight lowering of the concentration occurred. Only with low concentrations could the mean value occasionally turn out to be a little too high. In this case the correction gave an obviously better result. As to the figures, this implied a better fitting of the points at the lower concentrations, in the absorption curve. In view of the slight significance of the correction to the result mentioned below, it was omitted. Thus all absorption rates were plotted against the mean value of initial and final concentration. The calculations were also based on these values.

From a theoretical point of view, Langmuir's equation is of more importance. As to the relation between the rate of uptake u and the external concentration c, it is given by:

$$u = \frac{k_1 c}{1 + k_2 c}$$

It follows from this equation that there is a rectilinear relationship between the reciprocal values of absorption $1/u$ and concentration $1/c$. Thus, it can easily be found how far the data are in agreement with Langmuir's equation. The constants k_1 and k_2 follow immediately from the calculation of the straight line, for:

$$\frac{1}{u} = \frac{1}{k_1} \cdot \frac{1}{c} + \frac{k_2}{k_1}$$

A formula can be derived from Langmuir's as well as from Freundlich's equation, which gives the correct concentration, to which the uptake found belongs. The result is:

$$c = \frac{c_o - c_t}{\ln c_o - \ln c}$$

where c_t and c_o again represent the concentrations at the start and at the end of the absorption period.

Of great theoretical interest is the fact, that according to Langmuir's equation, if the external concentration is increased infinitely, a "saturation value" for the rate of uptake will be obtained. This value equals k_1/k_2. It is possible by this to compare the experimental saturation value with this theoretical one in those cases where a maximum uptake was obtained.

In all experiments mentioned below, both the Freundlich and the Langmuir equations were calculated. Along with the description of a few experiments, the difference between these equation as to their ability to represent the data, will be discussed. In practical aspects Langmuir's equation is preferable because it requires far less tedious calculations. The theoretical significance of this expression will be fully demonstrated in the discussion.

Experiment 4a. This first experiment was carried out with a set of twelve old low salt plants. The plants were vigorously developed. Moreover, the absorption period lasted for three hours. An enormous uptake took place, causing a large alteration of the concentration (fig. 5: A).

During the first day four solutions of increasing concentration were tested. The relation between the rate of phosphate uptake and the mean values of initial and final concentration can be represented by a Freundlich equation ($u = 1{\cdot}71\ c^{0{\cdot}476}$) or a Langmuir equation ($u = \frac{1{\cdot}36\ c}{1 + 0{\cdot}0144\,c}$). The two corresponding curves have been drawn in fig. 5: A. At the very low concentrations Freundlich's formula produces values a little higher than Langmuir's. The same applies to the high concentrations. It will be clear that it cannot be decided which curve gives the closest fit.

On the second day the experiment was repeated. Glucose was now added to every solution. There was an obvious enhancement of the phosphate absorption. An absorptive relation applied only to the first three concentrations. For this concentration range the equations give: $u = 1{\cdot}02\ c^{0{,}94}$, resp.: $u = \frac{0{\cdot}966\ c}{1 + 0{\cdot}0063\,c}$. It follows from the high value of the exponent of Freundlich's equation, that the curve was nearly rectilinear.

The alterations of the concentrations during the absorption periods were very large. The correct concentrations were calculated together with the Langmuir equation $u = \frac{1{\cdot}252\ c}{1 + 0{\cdot}0030\ c.}$ As is shown by fig. 5: A, the error was considerable. In the other experiments this was not found, as a result of the use of a smaller number (8—10) of young plants, which were only allowed to absorb for two hours.

From the Langmuir formula the saturation value was calculated. On the first day it amounted to 94 mg P_2O_5/3h., on the second day, when glucose was added: 153 mg P_2O_5/3h., resp. 417 mg P_2O_5/3h. As a matter of fact the uptake on the second day proved to rise not above about 14 mg P_2O_5/3h. This pointed to a limiting factor.

Experiment 4b. A set consisting of young high salt plants (June 1950) was used. On the first day the uptake on a full-strength Hoagland solution during four successive absorption periods amounted to:

$$0{\cdot}34 \quad 0{\cdot}30 \quad 0{\cdot}42 \quad 0{\cdot}30 \text{ mg } P_2O_5/2h.$$

This variability falls within the error of the phosphate determinations.

On the second day the uptake on six solutions of increasing concentration was studied (fig. 5: B). During the fourth absorption period aeration was insufficient. The immediate depressing effect on

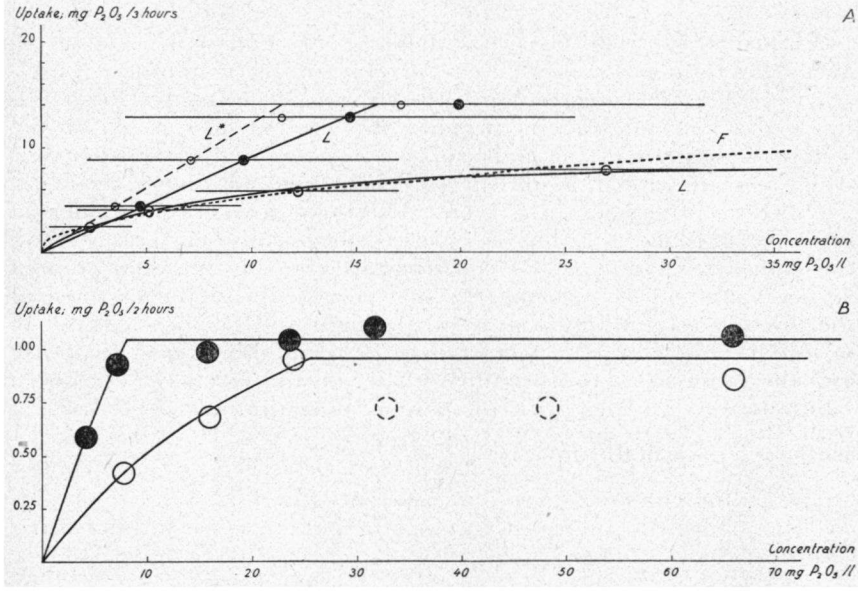

Fig. 5. The relation between the rate of the phosphate uptake and the external concentration, before (o—o) and after (●—●) glucose addition. A: by a set of large low salt plants; B: by a set of high salt plants. F: Freundlich curve; L: Langmuir curve; L*: corrected Langmuir curve, belonging to corrected concentration values.

the uptake was striking. During the fifth period the uptake had not yet attained its original rate. It is not very likely that the uptake would have been much higher during the sixth period, in view of the absorption rates found the next day. For the three lower concentrations we obtained: $u = 0{\cdot}097\ c^{0{\cdot}715}$, resp. $u = \dfrac{0{\cdot}0685\ c}{1 + 0{\cdot}0336\ c}$.

From the last expression a saturation value follows: 2·3 mg P_2O_5/2h. The maximum rate obtained was $\pm$ 0·95 mg P_2O_5/2h. that was already attained at a concentration of 24 mg P_2O_5/l.

The next day glucose was added to the solutions. For the two lower concentrations we obtained: $u = 0{\cdot}182\, c^{0{\cdot}829}$, resp.: $u = \frac{0{\cdot}162\, c}{1 + 0{\cdot}0316\, c.}$ In this case also, the relation was almost rectilinear after the addition of glucose. The saturation value was also much larger viz. 51·3 mg P_2O_5/2h. The uptake was found not to increase in practice any longer above a concentration of 8 mg P_2O_5/1. It did not differ significantly from the maximum rate of uptake the day before, when no glucose was administered. Hence, by addition of glucose the amount of phosphate that can be utilized maximally by the plant was not increased, although the ease with which phospate can be taken up from the external solution was increased.

Experiment 4c. This experiment was performed with a high salt and a low salt set, grown under very favourable conditions (July—August 1951). During the first two weeks of cultivation both sets received a high salt treatment. Then the low salt set was put on tap water, the solution of the high salt set being renewed every other day. After one and a half week the sets were used for the experiment. They were brought into the room of constant temperature under an intensive illumination. The day before the experiment was started, the high salt set was placed in darkness to reduce the sugar content as much as possible. During the first three days of the experiment the low salt set continued to be in the light and the high salt set to be in darkness. Figure 6: A represents the relation between the uptake and the concentration of the low salt set on the first day. The seven solutions were applied with increasing concentrations. There was a regular increase of the uptake ($u = 0{\cdot}273\, c^{0{\cdot}384}$, resp. $u = \frac{0{\cdot}432\, c}{1 + 0{\cdot}502\, c}$). Quite a different result was obtained with the high salt set on this day (fig. 6: B). At low concentrations the uptake rose with increasing concentrations ($u = 0{\cdot}471\, c^{0{\cdot}341}$, resp. $u = \frac{0{\cdot}632\, c}{1 + 0{\cdot}516\, c}$). But at a concentration of about 14 mg P_2O_5/1. the maximum rate of uptake was attained. At higher concentrations the uptake remained 1·12 mg P_2O_5/2h. The theoretical value derived from the Langmuir's equation, amounted to 1·22 mg P_2O_5/2h.

The next day this experiment was repeated. Higher concentrations up to 66 mg P_2O_5/1. were used. They were used in decreasing concentrations. Fig. 6: C and D represent the results (the absorption values at the higher concentrations have been omitted, in order to bring the figure into accord with the other ones). With the low salt set the uptake was again found to increase over the whole range of concentrations used ($u = 0{\cdot}457\, c^{0{\cdot}252}$). The rates were rather higher than they were on the first day. At the lowest concentrations the values were even higher than could be expected theoretically. This could be explained by assuming an increase in the absorption activity during the last two absorption periods on this day. For on the next day the uptake proved to be much higher than it was on the previous days.

The result for the high salt set on the second day was quite different to what it was on the first day (fig. 6: D). The absorption velocity was greatly decreased. Moreover, there was no maximum uptake. At the highest concentration (66 mg P_2O_5/1.) the rate of uptake was 1·06 mg P_2O_5/2h. (not represented by the figure). This equalled the maximum rate of the first day. Hence, the rate was not very likely to increase with still higher concentrations. Thus, by a prolonged stay in the darkness the maximum amount of phosphate was not diminished but the ease with which phosphate could be absorbed from low external concentrations was ($u = 0{\cdot}107\, c^{0{\cdot}542}$, resp. $u = \frac{0{\cdot}0765\, c}{1 + 0{\cdot}0812\, c}$). The efficiency of the absorption mechanism was diminished. The theoretical saturation value had also been lowered only little, it amounted to 0·94 mg P_2O_5/2h.

On the third day the influence of the addition of glucose was traced. As a check, the uptake was first determined on a Hoagland solution without glucose. After this the sets were placed on a solution of the same concentration to which glucose was added. Then four more solutions of decreasing concentrations, also with glucose, were used.

The uptake of the low salt plants (fig. 6: E) was much higher than it was the days before ($u = 1{\cdot}002\, c^{0{\cdot}179}$, resp. $u = \frac{1{\cdot}518\, c}{1 + 0{\cdot}849\, c}$). However, as will appear from a comparison with the control, this enhancement was not the result of the sugar supply. It must be due to a normal increase in the absorption activity of the low salt plants (see irregularities at the end of the second day). It is remarkable that this increase was obtained at all concentrations. The ratio between the rates of uptake on this day and the rate on the first day follows from the Freundlich equations. It amounts to $3{\cdot}7\, c^{-0{\cdot}2}$. This means that the lower the concentration the higher this ratio: i.e. the enhancement of the uptake. The absorption mechanism had become more efficient. This follows immediately from the falling values of the exponents of the Freundlich equations (0·384, 0·252, 0·179). From the Langmuir equations it can be seen that the saturation values were also raised (up to 1·78 mg P_2O_5/2h.).

With the high salt set the effect of the glucose supply was obvious (fig. 6: F). The effect increased during the course of the day and especially during the second part. The result was an irregular relationship between the concentration and the uptake: the uptake at a lower but later applied concentration could be higher. It is difficult to give the theoretical relationship but rather arbitrarily it is indicated in two ways viz. for the uptake at the higher concentrations (curve *a*) and for the uptake at the lower concentrations (curve *b*). The Freundlich equations were: $u = 0{\cdot}127\, c^{0{\cdot}608}$, resp. $u = 0{\cdot}633\, c^{0{\cdot}349}$.

The uptake without glucose at a concentration of $\pm$ 32 mg P_2O_5/1. was not significantly different from that of the previous day, so a continued decrease as a result of the dark treatment had not occurred.

Therefore, the relationship between the uptake and concentration may be assumed not to be changed very much, so that it can be represented approximately by the same curve (curve *c*). It appears from a comparison of curves *a* and *c* that by the addition of glucose the uptake was enhanced. The enhancement gradually decreased with the external concentration. The same result was obtained with experiment 4a and 4b (fig. 5). The Freundlich line was less curved.

Quite different conclusion can be derived from a comparison of curves *b* and *c*. The enhancement was much higher. This result can be compared with that of the low salt set of this experiment (a stronger curvature of the Freundlich line). The conclusion seems to be justified that the glucose had a complex influence on the phosphate uptake. How far the occurrence of the secondary effect (which was not obtained in the previous experiments) was connected with the different sequence in which the concentrations were used needs further examination. At any rate, the high salt plants showed a decrease of absorption in the darkness, especially at the lower concentrations. Probably the maximum uptake possible did not change very much. The uptake was enhanced by sugar supply. The effect increased during the course of the day.

At the end of these first three days of the experiment the low salt set was placed in the dark, the high salt set in the light. The nutrient solution of the latter set was renewed daily; the former one was put on tap water. This treatment lasted for three days. Then, on the sixth and seventh day of the experiment, the relation between uptake and concentration was once more determined; on the sixth day without and on the seventh day with addition of glucose.

Figure 6: G shows the result of the low salt set without glucose ($u = 0{\cdot}466\, c^{0{\cdot}325}$). The relationship between the concentration and uptake was virtually similar to that of the first day. The rates at all concentrations were 1·7—1·8 times those of the first day. There was a slight irregularity in the rate of uptake at 17 mg P_2O_5/1. which was a little higher than could be expected. The Langmuir curve ($u = \frac{0{\cdot}346\, c}{1 + 0{\cdot}224\, c}$) fitted much closer to the data than did the Freundlich curve. The theoretical saturation value was 1·54 mg P_2O_5/2h. Thus this showed a decline.

The result of the high salt set was more irregular, because of the increase in absorption activity during the course of the day. During the first three absorption periods the uptake rose in spite of the decreasing concentration. Figure 6: H shows the relation for the lower four concentrations ($u = 0{\cdot}941\, c^{0{\cdot}310}$). The maximum rate of uptake found (2·15 mg P_2O_5/2h). was attained at a concentration of 14·5 mg P_2O_5/1. This was very likely to be the highest possible uptake, as can be seen from a comparison with the results of the next day.

The addition of glucose had a very strong effect with the low salt set (fig. 6: *I*). The uptake had become very low as a result of the dark treatment (0·6 mg instead of 1·4 mg P_2O_5/2h.). The increase

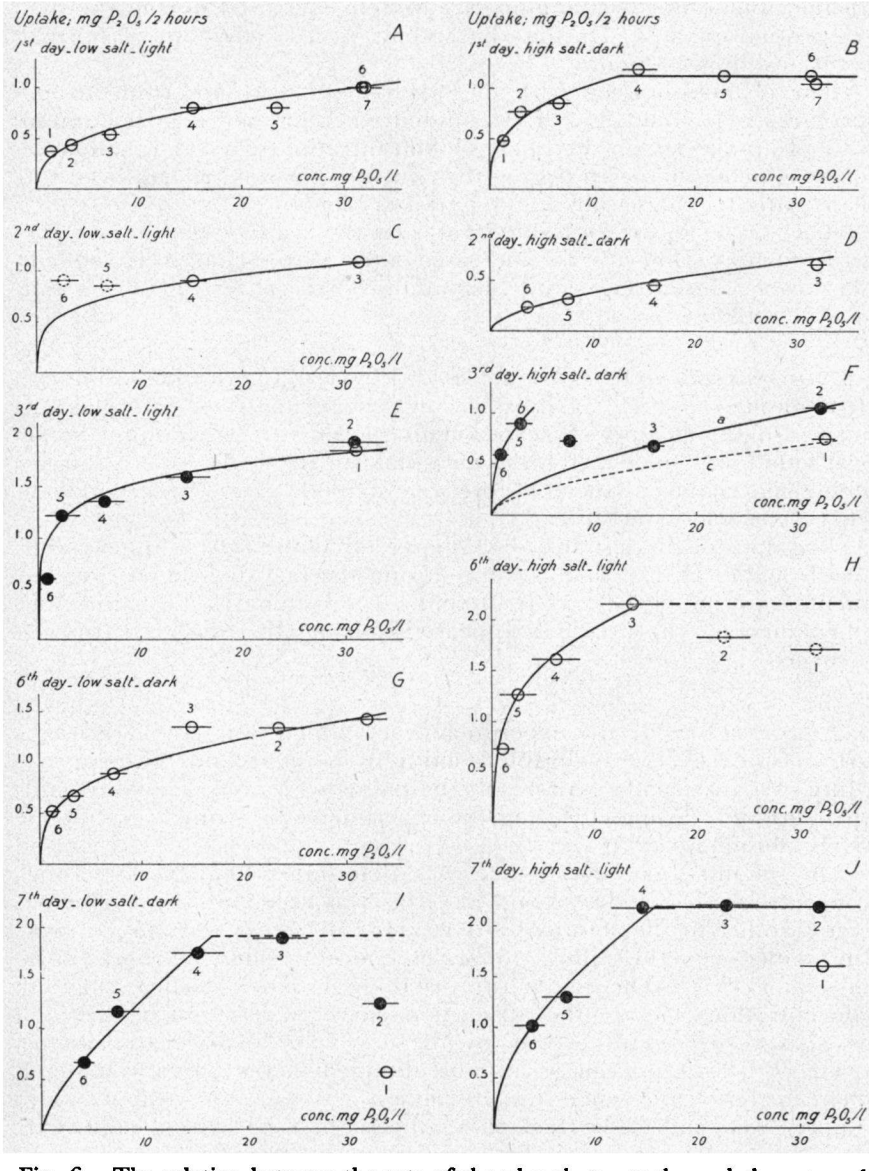

Fig. 6. The relation between the rate of the phosphate uptake and the external concentration with high salt and low salt plants, in light and darkness without (o—o) and with (●—●) glucose addition. In all graphs, Freundlich curves have been drawn.

of the uptake due to the glucose addition, increased during the first absorption periods. During the second part of the day a normal relationship was found ($u = 0{\cdot}259\, c^{0{\cdot}714}$).

The results obtained with the low salt set were according to expectations. It could not be established whether there is a maximum rate of uptake within the range of concentrations used. The influence of the glucose increased during the course of the day, as had been the case with the high salt set in darkness (fig. 6: F).

The uptake of the high salt set was on the last day less than on the previous day. The uptake increased after glucose had been added. At lower concentrations the equations were: $u = 0{\cdot}497\, c^{0{\cdot}537}$ and $u = \frac{0{\cdot}420\, c}{1 + 0{\cdot}151\, c}$.

A maximum absorption rate of 2·19 mg P_2O_5/2h. was attained. It amounted to 2·15 mg P_2O_5/2h. on the previous day. So it was not increased significantly. In experiment *4b.* also the maximum uptake was found only to change little, the uptake at the lower concentrations being markedly enhanced. Moreover, in both cases the Freundlich curve was more rectilinear.

The data of the last two days showed that the rate of uptake was much higher than it had become during the first days of the experiment as a result of the dark treatment. At the same time the saturation phenomenon, that had disappeared during the dark treatment, returned.

Experiment 4d. In this experiment a set of high salt plants was used, grown under very favourable conditions of light and temperature (July—August 1950). As a result the plants were well developed and their uptake was high. During the experiment the plants were intensively illuminated.

On the first day the uptake was determined for six increasing concentrations. On the second day this was repeated, 2 gm glucose being added to the solutions. As figure 7: A shows, the uptake was the same over the whole range of concentrations on both days ($u = 3{\cdot}33\, c^{0{\cdot}190}$). The uptake rates were large, particularly at the low concentrations (where the absorption curve is very much curved), as is expressed quantitatively by the low value of the exponent of c, viz. 0·190. A simular result was obtained in the previous experiment in those cases where the absorption activity, also without sugar supply, was high. The fact that glucose had no effect, indicated that the plants were in a high sugar condition. The absence of a maximum absorption rate is particularly striking, though very high concentrations were used. Thus there was no limiting factor with these rapidly growing plants.

Figure 7: A also shows the Langmuir curve ($u = \frac{1{\cdot}692\, c}{1 + 0{\cdot}230\, c}$). Its closer fit to the data, especially at the lower concentrations, is remarkable. This was a normal result with plants showing a high

absorption rate also at low concentrations. This is connected with an essential difference between the two types of curves. The curvature of the Freundlich curve continues to increase with lower concentrations. It attains its maximum at $c = o$. This does not apply to Langmuir's equation. The concentration at which its curvature is highest, follows from: $c = (k_1 - 1)/k_2$. Depending on the value of k_1, this concentration may be either negative, zero or positive. The last possibility is significant as it gives a picture differing from Freundlich's curve. In figure 7: A the Langmuir curve shows its highest curvature (for the given method of plotting) at 7·0 mg P_2O_5/l. The relationship is more rectilinear below this value as well as above this value. In principal by experiments such as this one, a decision is rendered possible as to which equation, Freundlich's or Langmuir's, gives the closest fit to the data.

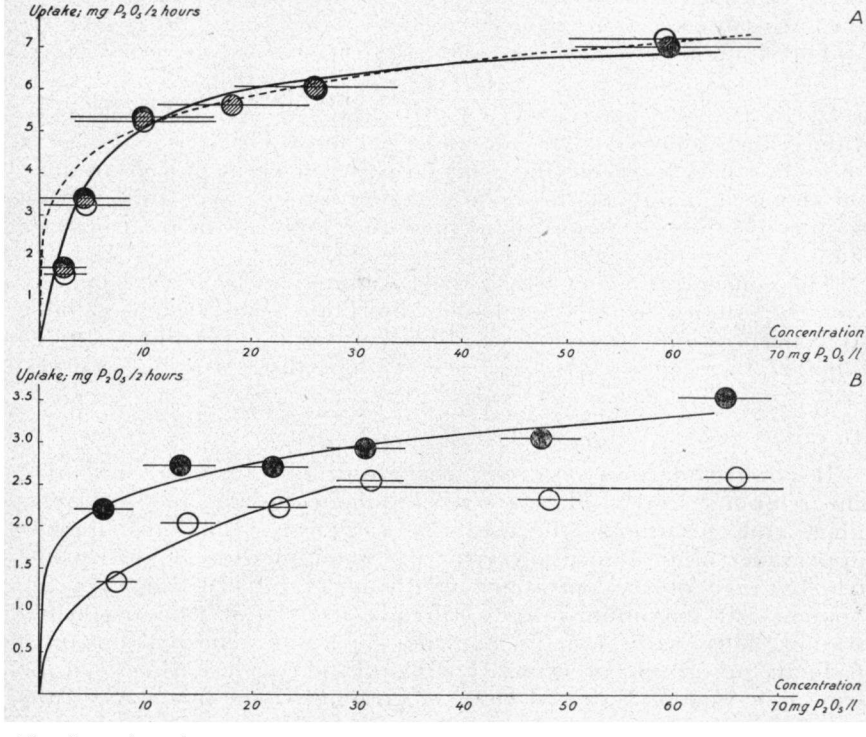

Fig. 7. The relation between the rate of the phosphate uptake and the external concentration before (o—o) and after (●—●) glucose addition. A: high salt plants; B: low salt plants.

Experiment 4e. A few weeks after the previous experiment a similar experiment was carried out with a low salt set. The results are represented by figure 7: B. Solutions were applied with increasing concentrations, on the first day without and on the second day with glucose. The uptake seemed to be stimulated by glucose addition.

But it is quite possible that it was the normal increase of absorption activity as had been generally found with low salt material (e.g. experiment 4*c*; fig. 6: E). Moreover, sugar supply was not very likely to be a limiting factor with low salt plants, which normally are in a high sugar condition. The most striking result was the finding of a concentration above which there was no further increase of the phosphate uptake. This result was no longer obtained on the second day, when the absorption activity had been increased. This suggests that the saturation phenomenon on the first day was due to some physiological factor allowing only a restricted uptake. This limitation was removed the next day. This renders the factor likely to be connected with the low salt condition. The most striking feature of the low salt plants, in contrast with the high salt ones, was their inhibited growth particularly in the shoot. Hence, as a preliminary explanation of the saturation effect, the limiting influence of the growth, due to the low salt condition, may be used.

The Langmuir equations were on the two consecutive days: $u = \frac{0{\cdot}327\,c}{1 + 0{\cdot}097\,c}$ and $u = \frac{1{\cdot}033\,c}{1 + 0{\cdot}310\,c}$. From these the saturation value can be derived: 3·40 mg and 3·33 mg P_2O_5/2h. No change of this value had occurred. But whereas this value was indeed attained on the second day at the highest concentration used, this did not happen on the first day. This pointed once more to the omitting of a limiting factor.

The concentrations at which the Langmuir line was most curved, were 6·4 and 6·0 mg P_2O_5/1. No absorption value being available at concentrations below these values, it was not possible to judge whether these curves gave a closer fit than the Freundlich curves.

Discussion

If no irregularities occurred, the absorption curve, representing the relation between the rate of phosphate uptake and the phosphate concentration of the medium, was always found to have the appearance of an adsorption-isotherm. Sometimes this applied to the whole range of concentrations used (up to 60—70 mg P_2O_5/1.). Sometimes a maximum rate was already attained at a lower concentration. This "saturation phenomenon" is quite a normal one with ordinary adsorption processes. Therefore, the conclusion seems to be legitimate that, as VAN DEN HONERT expounded, the first stage of the uptake consists of an adsorption process, by which equilibrium is rapidly established. The ions adsorbed in this way have to be transported further at a constant velocity. Therefore, to the whole process of uptake the 'picture of the conveyor belt can be applied.

But, though the general relation between concentration and uptake becomes understandable in this way, it is not possible to explain all the facts obtained in these experiments. For instance it was found that glucose supply enhanced the uptake. This might be explained by assuming the glucose to be partly phosphorylated. However it is

then difficult to see why the relationship between the concentration and the resulting uptake (now consisting of the normal uptake and the utilization by phosporylation) has again an adsorptive nature. Also the fact, that sometimes no influence at all was found of the glucose supply at the higher concentrations, speaks against this assumption. It must be assumed that the normal uptake has been increased. To use the picture of the conveyor belt: either the loading of the belt or the rotation velocity has been altered. It is obvious first to think of the rotation velocity, as it is closely connected with metabolism. But if this is correct, the uptake can be expected to be enhanced relatively to the same extent at every concentration. The relation between uptake and concentration being represented by $u = k.c^{1/n}$, only a change of k can be expected as a result of the addition of sugar. Such an alteration of the relation was found by van den Honert as to the influence of temperature. Moreover as far as a maximum rate of uptake had occurred, this should have been found to happen at the same concentration.

As a matter of fact the uptake was always found to change in a different extent at different concentrations. Such a change not only occurred as a result of glucose addition. By a dark treatment the uptake was diminished especially at the lower concentrations. Low salt plants showed an increase in the absorption activity during the first days of the experiments. This increase happened again mainly at the lower concentrations. On the other hand, if the uptake was enhanced by glucose addition, the higher concentrations were favoured. The relation became nearly rectilinear.

This means it had to be assumed that the first stage of the uptake: the "adsorption" (the loading of the conveyor belt) can be changed too e.g. by glucose addition. This consideration can easily lead to the hypothesis that this first stage involves a formation of hexosephosphate. At any rate, the experiments with phloridzin pointed to the fact that phosphorylations are significant in the uptake of phosphate. If this hypothesis is correct, it means that the first stage of the phosphate absorption by plant roots is enzymatic in nature. For many enzymatic processes it is known that the relation between the reaction velocity and substrate concentration can be represented by a Langmuir equation. This is explained by the theory of MICHAELIS and MENTEN (HÖBER 1945; SCHWAB 1941).

The hypothesis, that the phosphate uptake begins with the formation of a hexose phosphate, was the very starting point of the exposition given below. Meanwhile, this hypothesis did not prove to be a necessary condition to give an explanation of the phenomena obtained. For this it is sufficient to generalize the picture of the conveyor-belt followed till now. Meanwhile, the strong resemblance between the Langmuir treatment and the Michaelis-Menten formulation of enzyme kinetics is evident from this (BULL 1951). This need not surprise us since the Langmuir equation has a very general form so that it applies to various processes and reactions (HINSHELWOOD 1946). For a critical discussion of the principles, involved in

catenary processes and limiting factors, reference may be made to an excellent survey of BOOY and WOLVEKAMP (1944).

Phosphate P, present in the medium, is assumed to be bound in a reversible way to a substance X, which is present in the symplasm of the root only in a limited amount. This process can be represented by:

$$P + X \underset{k''}{\overset{k'}{\rightleftarrows}} PX$$

It should be added that this representation may be a simplified expression of a more complicated chain of reactions. The "velocity constants" k' and k'' may appear to depend on many factors e.g. on the sugar metabolism. Moreover X may also represent some protoplasmic particle.

Secondly phosphate must be transferred at a constant rate. It may be that the complex PX is immediately broken down. However, it is equally probable that phosphate is transferred as PX and that the decomposition occurs after some time. In both cases some X becomes again available for binding phosphate of the external solution. Attention should be drawn to the fact that phosphate can be liberated from PX into the medium as well as into the symplasm of the root. The former process will be referred to as dissociation of PX. The latter process constitutes the uptake and will be referred to as transfer of phosphate. The rate of this transfer (it is the rotation velocity of the conveyor belt) can be expected to be dependent on the glucose concentration. As to the velocity u with which phosphate is absorbed:

$$u = k\, c_{PX} \cdot c_G$$

now applies, where c_{PX} and c_G represent the concentrations of the compound PX and of glucose.

During a steady state the concentration c_{PX} has a constant value. The velocity with which the compound is formed then equals the velocity with which it is broken down by dissociation and transfer. Hence:

$$k' c_P c_X = k'' c_{PX} + k\, c_G c_{PX}$$

If the total available amount of the substance X equals C, so that:

$$C = c_X + c_{PX}$$

the concentration of PX can be derived from the two last formulae:

$$c_{PX} = \frac{k' C\, c_P}{k'' + k\, c_G + k' c_P}$$

Substitution of this value of c_{PX} in formula concerning the absorption rate gives finally:

$$u = k' C\, k\, c_G \frac{c_P}{k'' + k\, c_G + k' c_P}$$

This expression can easily be written in the general form of Langmuir's equation:

$$u = \frac{k_1 c_P}{1 + k_2 c_P}$$

if:

$$k_1 = \frac{k\, k'\, c_G\, C}{k'' + k\, c_G} \quad \text{and} \quad k_2 = \frac{k'}{k'' + k\, c_G}$$

The maximum possible rate of uptake k_1/k_2 now tends to $k\, c_G\, C$ This could be expected, since this rate of uptake only occurs if the whole available amount of substance X is continuously bound to phosphate, so that c_{PX} equals C. This saturation value is always proportional to C. The same applies to the absorption velocity at lower concentrations. However, the glucose concentration has quite another influence. The uptake is increased by glucose addition, it is true, but the extent to which this happens depends on the external phosphate concentration. This can be illustrated most clearly by considering two extreme cases, each of which can be represented by a more simple expression.

In the first place the transfer of phosphate ($= k\, c_G c_{PX}$) can be supposed to be negligible compared with the dissociation velocity of this compound ($= k'' c_{PX}$). Then $k c_G \langle\langle k''$ and the formula becomes:

$$u = k \frac{k'}{k''} \cdot c_G \cdot C \frac{c_P}{1 + \frac{k'}{k''} c_P}$$

Increasing the glucose concentration has the same effect that increasing the X concentration has viz. the same enhancement at all phosphate concentrations. It produces the same result that could be expected on the grounds of the initial theoretical considerations. This is understandable, for these considerations lead to the same equation. For if the first stage of the uptake consists of an adsorption, the equilibrium of which is established very rapidly compared with the rate of further transport, the "loading of the conveyor belt" is given by:

$$c_{PX} = \frac{k'}{k''} \cdot C \frac{c_P}{1 + \frac{k'}{k''} \cdot c_P}$$

if Langmuir's derivation is used. Now C represents the total quantity of adsorption-points, c_{PX} the concentration of the points occupied by phosphate ions. In order to obtain the formula for the uptake, the expression needs only to be multiplied by $k\, c_G$; i.e. the rotation velocity of the conveyor-belt.

The second extreme case occurs if the dissociation velocity of PX is low compared with the velocity at which phosphate is transferred

($k'' \langle\langle k\, c_G$). The formula can be simplified to:

$$u = k'\, C \frac{c_P}{1 + \frac{k'}{k'' c_G} \cdot c_P}$$

Increasing the glucose concentration involves also in this case an enhanced phosphate uptake. This enhancement is however different at different concentrations. The range of concentrations over which the uptake is nearly linearly proportional to the external phosphate concentration becomes much larger after glucose addition. This is more in agreement with the experimental results. With glucose the relation between uptake and concentration was virtually rectilinear; this was clearly demonstrated by the high value of the exponent of the Freundlich equations.

This second case fits more closely to the data. But this does not mean, that the extreme conditions of this case were always fulfilled. The result indicated will also be obtained with less extreme conditions, viz. as long as the absorption velocity of phosphate remains a non-negligible factor compared with the dissociation velocity of the complex *PX*.

As a starting point for the foundation of this theory the hypothesis was used that phosphate was bound initially to glucose. It will be clear that the proposition, just given, can be applied to this case unaltered. The role of the substance *X* can be played by either some enzym or some intermediate product produced at the formation of hexose phosphate. It is tempting to think particularly of the energy-rich phosphorus compounds. By this the results with low salt material might be partly explained. For, by supplying phosphate during the experiments they are allowed to enlarge their adenyl acid-complex. By this the phosphate uptake might be enhanced immediately. Moreover, the check on the development of the plants might be abolished and this will have a secondary effect on the phosphate uptake i.e. by removing the limiting effect of growth on the uptake at high concentrations.

Attractive as this hypothesis may be from a biochemical point of view, the general concept should be preferred for the present. A further elaboration needs more biochemical examination. A great advantage of the exposition as given here, is that it can be applied to other ions. It may be considered therefore, a quantitative elaboration of those theories in which ions are assumed to be bound to carriers.

If the explanation given is correct, Langmuir's equation proves to be of particular significance. It may be used in calculating the maximum uptake (the "saturation" value). This is of primary importance to those experiments in which a concentration was found above which the uptake did not increase any longer. Summarizing the following results were obtained:

Experiment	Treatment	Maximum uptake theoretical	Maximum uptake experimental
fig. 6: B, H, J high salt	darkness	1·22	1·12
	light	2·39	2·15
	light + glucose	2·78	2·19
fig. 5: A high salt	light	2·30	1·00
	light + glucose	51·3	1·04
fig. 7: B low salt	light	3·40	2·48

In the experiments with high salt material as a rule a maximum was found. Only as a result of a prolonged dark treatment was the uptake decreased and did the "saturation phenomenon" disappear. As is clear from the table, this experimental maximum level was mostly lower than the theoretical one. This means that there was another process, that may have a limiting effect on the uptake. The sugar supply might be supposed to be this limiting factor. This is disproved by the experimental data. By sugar addition the maximum uptake was not increased, although the uptake at lower concentrations was. A much higher uptake should have been expected, if the limitation was the result of a "saturation".

If neither the phosphate concentration nor the glucose supply can be the check on further increases of the phosphate supply, this check has to be sought in the further utilization of phosphate in the plant. With high salt plants the ability to accumulate ions in the vacuoles is but slight in contrast with low salt plants. This renders it intelligible why a maximum uptake is so often found with high salt plants. A more important factor than the vacuole accumulation is perhaps formed by the consumption of phosphate by growth processes.

In the experiments last mentioned in the table, glucose addition did not change the maximum absorption rate. This would mean that the growth processes were not enhanced by the glucose, but the phosphate uptake was, at least at low concentrations. On the other hand, with experiment 4*c* (fig. 6: F) clear secondary effects were observed on plants which had been in darkness for some days. Whether the maximum uptake was also increased by adding glucose, could not be affirmed. Meanwhile it will be clear that glucose may have a complex influence.

With low salt plants it was observed only once, that the uptake was limited at high concentrations in the same way as it was with high salt plants. This happened only on the first day of the experiment. At that time the development of the plants was as yet much inhibited by a phosphorus deficiency. Thus a limiting influence of the growth can also explain the "saturation phenomenon" in this case.

This "saturation phenomenon" has been the very starting point of this investigation into the relation between the phosphate uptake

and the external phosphate concentration. An attempt was made to study the influence of glucose on this maximum value in detail. Indications were obtained that it is possible to influence this phenomenon. It was assumed that this was due to a glucose effect on growth processes. How far the hypothesis is correct that there may be, in general, a regulating influence of utilization on uptake, will be discussed in the next section, on the basis of experiments specially planned with this problem in mind.

5. The direct influence of the utilization on the uptake of nitrate

In the preceding chapters the growth was occasionally supposed to be a regulating factor on the phosphate absorption. Along this line of thought moved Alberda and Van Andel, Arisz and Helder (1950). Starting from the assumption that high salt material is saturated with salt, it seems to be imperative that there must be growth before any uptake can occur. Also among the literature, particularly in old physiological and agronomical papers, the conception is met with of a regulating influence of the utilization on the uptake (Sachs 1874; Pfeffer 1897; Nelson 1946; Williams 1948), as was discussed in detail by the author. (Helder 1951) This idea is mainly supported by the frequent appearance of a correlation between growth and absorption activity (Steward 1935). There are however many factors by which the uptake is determined. In the case of the uptake of phosphate, the concentration of the medium, as well as the sugar supply, proved to be of great significance. Hence it is not entirely excluded beforehand that the uptake should be determined by these factors. Likewise a slight growth capacity could go together with a low content of sugar, which should mean a small uptake at the same time. It is true, it was rendered likely in the previous section that concentration and sugar supply are not the only determining factors, but the principal possibility obviously illustrates that the significance of the growth for the uptake is not so self-evident as is usually accepted. Moreover it follows that only by a direct causal analysis can it be settled how far the correlation between growth and uptake is the result of a determining influence of the growth on the uptake. This means in the first place that the plant material has to be in a condition at which a regulating influence of the growth on the uptake can be expected. The plants were therefore brought into a high salt condition. In the second place with this material it must be possible to regulate the growth. This was attempted with the aid of indole acetic acid, which was added to the nutrient solution. This had no effect however. Only at the highest possible concentration was the phosphate uptake inhibited (Schuffelen 1948). But then clearly damage occurred: the solutions were brown in colour at the end of the absorption period. In view of the results obtained, this negative result is not surprising. For, by this growth substance, in the first place the extension growth is inhibited. As we shall see it is rather the

protoplasmic synthesis that is connected with the uptake of anions.

Following on a suggestion from Prof. Dr W. H. ARISZ, use was made of the inhibiting effect of a lack of nitrogen or phosphorus on the growth. For this purpose an orientating experiment was made (1949) in which plant material was grown on a Hoagland solution, without nitrate or without phosphate. After about four weeks the plants were placed on a full-strength nutrient solution and the uptake of the phosphate, resp. the nitrate was determined. This experiment was performed under glasshouse conditions. As a result of this the course of the uptake was irregular. In spite of this there was an enhancement of the uptake of the nitrate owing to the addition of phosphate with the low phosphate sets within two days.

As, in the first place we were concerned with the principle of a regulating influence of the growth on the anion absorption, the definitive experiments of 1950 were performed with high nitrate, low phosphate material. In the first experiment the effect of administration of different quantities of phosphate on the growth and the uptake of nitrate was investigated. In the second experiment the number of treatments was enlarged and amongst others, phosphate was withheld once more and the effect was studied. With the nitrate absorption by high nitrate- low phosphate plants a result was more easily attained than with phosphate absorption by high phosphate-low nitrate material; this may be the result of a combination of two circumstances. In the first place nitrate is consumed by the growth processes (synthesis of proteins) to a greater degree than is phosphate. In the second place various phosphorus compounds of the cytoplasm have a more regulating influence on the metabolism than have the nitrogen compounds. In spite of this it must be possible to find some effect of the nitrate on the phosphate absorption with the right treatments and under the right conditions. And as a matter of fact Scott Russell and his collaborators seem to have obtained results with this tendency (personal communication).

Experiment 5a. The first experiment was performed with six sets of plants grown in June 1950. The six sets were divided into three groups (fig. 8: I, II and III). The plants were illuminated continuously. During the first two days the three sets remained on a solution without phosphate, the solution being renewed every day. Growth was slightly enhanced, probably due to the favourable circumstances in the room of constant temperature, since it was found that if this treatment was continued (fig. 8: A I) the development of the shoot gradually decreased; the increase in length as well as the number of young leaves appearing, decreased more and more. If however, only a small amount of phosphate (6.5 mg per day) was added, this decrease did not occur. The increase in length as well as the number of young leaves appearing remained more or less at a constant level (fig. 8: A II). A yet more vigorous development could be obtained by putting the plants (fig. 8: A III) on a solution with the normal amount of phosphate (a complete Hoagland solution).

At the end of the experiment phosphate was also supplied to the first set, which gave a striking effect: a rise in the increase of length and suddenly a larger number of new leaves appearing. The differences in development of the shoot due to the differences in amount of phosphate applied, were much more striking than could be repre-

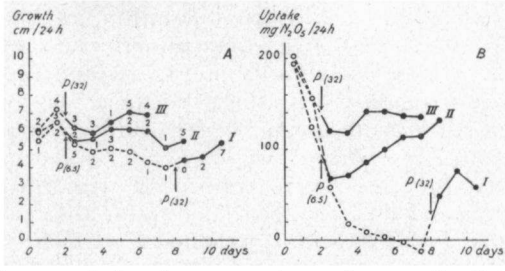

Fig. 8. The influence of phosphorus supply on the growth of the shoots (A) and the nitrogen absorption (B) of three sets (I, II, III) of phosphorus deficient maize plants. The curves are dotted during the time the plants were standing on a solution without phosphate. P (6,5) and P (32) refer to the amounts of phosphorus (mg. P_2O_5/l.) present in the nutrient solution. The growth is represented by the average increase in length of the youngest visible leaves and the number of new leaves appearing.

sented by the figure. It was already possible to distinguish on the 4th—5th day (that is 2 or 3 days after applying phosphate) the three groups by sight. The more phosphate that was administered the more vigorously were the shoots developed, while the colour changed from the dark green of plants with phosphate deficiency to a more yellow-green.

Quite the same but more striking results were obtained with the nitrate absorption. If no phosphate was supplied, there was a continuous fall in the rate of uptake, which came to a standstill after about five days (fig. 8: B I). If however, a small amount of phosphate was added to the solution each day, this fall was reversed, with some retardation, into a gradual increase of the rate of uptake. At the end of the experiment about the same level was reached as that of the third set, to which the normal amount of phosphate was supplied. With this set there was already some effect on the first day; then there was a slight rise up to a more or less constant level. Again a striking effect was obtained by phosphate supply with set I at the end of the experiment.

Owing to the low phosphate condition of the plants and the favourable conditions, a strong absorption of the phosphate occurred. During the first days the solution was completely exhausted. Thus the phosphate (0, 6·5 and 32 mg) added to enhance the growth was completely taken up. Only with the third set did a small amount of phosphate (about 1 gm) remain in the solution on the fourth and fifth day.

By this experiment the inhibiting effect of phosphorus deficiency on growth as well as on nitrate absorption was clearly illustrated.

By means of supplying phosphate this decline in both processes was immediately changed into an increase.

Experiment 5b. This experiment was performed with four sets of plants which were also in a high nitrate- low phosphate condition. Moreover two sets were examined, which were in a complete low salt condition (August 1950). The results are shown by fig. 9. Each of the separate graphic representations corresponds to one of the six sets used. By this the irregularities, particularly those of the growth are rather more large than in the former experiment, where the means of two sets were represented. The plants were illuminated for 16 hours every day.

There were three periods of four days during this experiment; each period being characterized by a certain treatment. During the first period all high nitrate sets (fig. 9: A, B, C, D) were on a Hoagland solution without phosphate. There was again a slight decrease in the growth but an obvious effect on the uptake; at the end of this period the absorption had almost come to a standstill.

Set A was hereafter placed on tap water and then for another period of four days once more on a solution without phosphate. So this set had not been able to absorb any phosphate from the medium either during the pretreatment or during the experiment itself. The depressing influence of the phosphate deficiency on the growth was clearly shown by the continuous decrease in the development of the shoot.

As to the nitrate absorption, during the tap water period no uptake could take place of course, but if then the plants were again put on a solution with nitrate, a renewed though small uptake appeared in spite of the fact that no phosphorus had been taken up. The amounts of nitrate taken up in this last period, are too large and the uptake goes on too long for this uptake to be due to an exchange. In this connection it may be recalled that the nutrient solutions were made with tap water. It can be concluded, that the standing still of the uptake at the end of the first period is not due to a direct depressing influence on the absorption mechanism, but rather to a nitrate saturated condition of the material. If only a slight growth had taken place, some uptake was again possible.

Quite another picture was obtained if a small quantity of phosphate was supplied (fig. 9: B). Compared with A, growth was only a little promoted; at the same time uptake started again and gradually increased. If phosphate was withheld once more, the development of the shoot diminished. Although the uptake of nitrogen went on for another two days, this was followed by a rapid fall. It could be proved by supplying some phosphate after this third period, that this fall was a consequence of lack of phosphorus. A rapid rise in the nitrogen uptake was then obtained (not reproduced in fig. 9: B).

If the amount of phosphate was increased to the normal level, stronger effects were obtained (fig. 9: C). There also was a vigorous further development during the third period, when phosphate was

again withheld. The uptake rose at once to a more or less constant level. During the third period the uptake even increased a little, the cause of this is not quite clear. Perhaps it was only an after-effect of the abundant phosphate supply. At any rate there was no fall as with the former set. The amount of phosphate supplied had evidently been sufficient for a vigorous further development.

Phosphate was also supplied to the last high nitrate set but at the

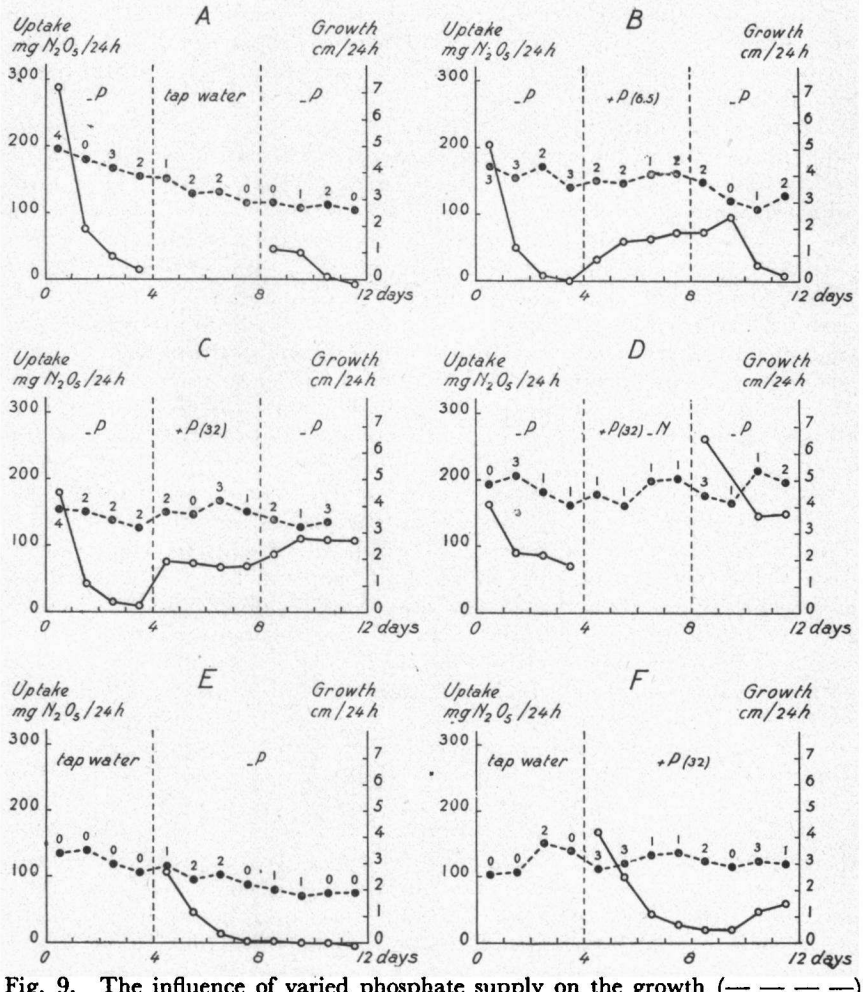

Fig. 9. The influence of varied phosphate supply on the growth (— — — —) and the nitrogen absorption (————) of maize plants. Set A, B, C, and D were at the start of the experiment in a low phosphorus, high nitrate condition; set E and F in a low salt condition.

Different media were used; tap water; a complete Hoagland solution (+ P 32); a solution with a reduced phosphate concentration (+ P 6,5); a solution without phosphate (— P) and a solution without nitrate (+ P 32, — N). All nutrient solutions were made with tap water.

same time however, nitrate was withheld (a combination of the treatments A and C). The growth and the uptake of this fourth set during the first period of the experiment was already rather different from the first three sets, particularly as there was a smaller number of young leaves appearing. This was the reason why A, B and C were used for the three main treatments. In spite of this drawback the results for this fourth set were quite clear. After supplying the phosphate, growth took place almost the same as it did with set C. From this it appears that this promotion of the growth was not the result of a renewed nitrate uptake. Nor could this be expected with this nitrate saturated material. If the set was then placed on a solution with nitrate but without phosphate an enormous uptake of nitrate took place, the rate was even higher than it was at the beginning of the experiments. Afterwards there was a slight fall, but the rate of uptake remained at a high level. This result once more points to the indirect influence of the phosphate supply on the absorption of nitrate.

The two low salt sets remained on tap water during the first period of 4 days. Then one set (fig. 9: E) was placed on a nutrient solution without phosphate. In spite of the fact that important ions were now being administered there was as with set A, a gradual fall in the growth. At the start of the experiment the low salt material was less strongly developed than were the high nitrate- low phosphate sets. This has to be ascribed to the lack of important ions like nitrogen and potassium. During the experiment however, in the first place phosphorus has a limiting influence on the development. By this it is possible to explain why results were obtained more easily in this way with high nitrate- low phosphate than with high phosphate- low nitrate material. The course of the nitrate uptake is also comparable with that of the high nitrate sets in that after some days the uptake comes to a standstill. In this respect these low salt plants can be compared with excised low salt root systems. As HOAGLAND and BROYER found (1936), low salt roots soon reach their maximum salt concentration. They ascribed the cessation of the absorption to the limited capacity of the roots for constructive metabolism associated with growth. This limited growth might be due to a deficiency of some elements e.g. nitrogen and phosphorus.

The second low salt set, which was placed on a complete Hoagland solution, also showed a rapid fall, but the uptake did not stop: after some time there was even a slight increase. This can only be the result of the growth, promoted by the phosphate supply.

Discussion

The results have already been amply discussed by the author (HELDER 1951). Hence a few remarks concerning the essentials will suffice.

In the first place it appeared that during the experiment the lack of phosphate especially depressed the development. By supplying phosphate this depression could be released. If a large quantity of

phosphate was administered the vigorous growth continued, even when phosphate was once more withheld.

At the beginning of the experiment all sets still showed a vigorous nitrate uptake which however decreased rapidly and stopped if no phosphate was added. That this was due mainly to the as yet unsaturated condition of the material, appeared from the similar behaviour of low salt and high nitrate- low phosphate material. During the pretreatment the solutions of the last kind of material were renewed twice a week. Apparently this does not suffice to bring the material into a high nitrate condition, because for that it is necessary to renew the solutions diurnally, as was done during the experiment.

Phosphate being withheld, growth was inhibited more and more and the uptake of the nitrate came virtually to a standstill. This standstill of the uptake proved not to be the result of a direct influence of phosphate deficiency on an active absorption mechanism (compare DAVIES and OGSTON 1950), but of the saturated nitrate condition. If nitrate was not supplied for a few days, some nitrate could afterwards be taken up again as a result of the continued, though very slight growth. A real enhancement of the uptake was only found if phosphate was supplied, which removed the factor depressing the growth. It is a well-known fact that phosphorus is of great importance in the synthetic processes of the cytoplasm (NELSON 1946). The protein synthesis is especially depressed by a phosphorus deficiency. An accumulation of soluble nitrogen compounds and sugars is connected with this (WILLIAMS 1938; RICHARDS and TEMPLEMAN 1936; GREGORY 1937). Slightly different results were found at first by SCOTT EATON. Later however he obtained results which were more in agreement with current notions (SCOTT EATON 1949, 1950). So in the first instance the synthetic processes are stimulated by phosphorus supply, and nitrate ions are utilized. Strictly speaking these experiments showed that under certain conditions the utilization of nitrate ions can be a determining factor for the absorption. These synthetic processes however, are so intimately connected with the formation of protoplasm that it seems justified to speak of growth of protoplasm and to hold this responsible for the nitrate uptake. Increased formation of protoplasm as a result of the administering of phosphate leads to increased meristematic activity. (NELSON 1946; BURSTRÖM 1947). Further, this increased rate of cell division, leads after cell elongation to the promoted, outwardly perceptible growth. As far as roots are concerned, this means at first an enlarged absorbing surface. In the second place, as a result of cell elongation, a renewed accumulation in the vacuoles of root and shoot is made possible, by which an increased uptake is also made possible. Therefore a close relation between this growth, which depends on cell elongation and uptake which is mainly determined by protoplasmic formation cannot be expected. Under these circumstances it is a question of taste if one prefers to speak of the growth as a determining factor for the uptake, or not. Whenever this might give rise to any misunderstanding, utilization will be used instead of growth.

Meanwhile it will be clear why the results obtained concerning the nitrate absorption were more clear cut than those concerning growth, because the growth measured was mainly based on cell elongation and this need not stop immediately if protoplasmic growth has become very slight. Moreover, only the growth of the shoot was measured. Not is this only rather a crude index of the development but it is also a not very obvious measure of the quantitative significance of the root growth.

6. The course of the phosphate uptake in light and darkness

As was already stated in the introduction, the starting point of these experiments, was the continuation of Alberda's investigation (1948) into the influence of light and darkness on the course of phosphate uptake. In particular it was important to experiment with low salt plants, so that the results of both types of material (low salt and high salt) can be mutually compared.

In principle it now seems possible to interpret the results of these experiments with the aid of the points of view put forward in the preceding sections. Firstly, the sugar supply proved to be of primary significance to the phosphate uptake. Next in importance to the sugar supply is the salt condition. Taking into account these two conditions and the various processes being important to the uptake, an explanation was attempted of the course of the uptake in light and darkness. In order to judge the correctness of the explanations, material was harvested and analysed from different moments in the experiments. As will appear from the analytical results, variability was too large to render possible any decisive conclusion. In spite of this a possible explanation will occasionally be given with the description of the experiments. Of course, it must be stressed that these explanations are of a hypothetical nature.

Because the sugar supply is an important factor for the phosphate uptake, it need not surprise us that the light intensity used in these experiments has an influence on the results. As a matter of fact, the results obtained during the first year of experimenting (1948) when a moderate illumination was used, differed slightly from those obtained during the next year, when high light intensity was available. The experiments will be discussed in two corresponding groups.

a. Experiments with moderate light intensity

The experiments of 1948 were performed with an illumination of fluorescent lamps (fig. 1: A). Moreover, the plants were standing in the case used for the experiments concerning the influence of carbon dioxide-free air on the phosphate uptake. The case was not closed. But with the open case and this low light intensity, the development of the plants was less prosperous than during the next year, when the case was removed and a high light intensity was used.

In the first place the different behaviour of low salt and high salt material was studied. It appeared that there is only a relative differ-

ence between high salt and low salt material. Hence the influence of light and darkness on low salt plants was examined more closely. Solutions of various concentrations were used, allowing the low salt sets to change into a high salt condition variously rapidly.

Experiment 6a. To be able to compare low salt and high salt plants, three sets of plants of low salt as well as high salt were grown at the same time (June—July 1948). At the start of the experiment they were 6—7 weeks old; that is a little older than was the case for the bulk of the experiments. The plants were slightly taller, and as a result of this they were hindered more by the limited space of the case. The solutions were renewed every twelve hours and the uptake was

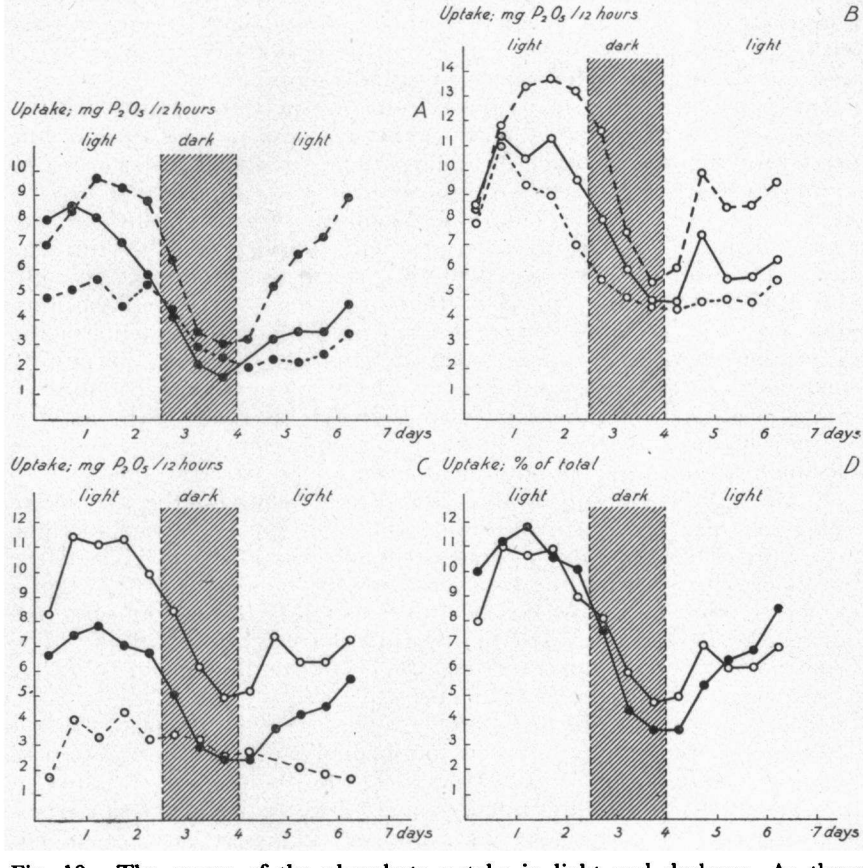

Fig. 10. The course of the phosphate uptake in light and darkness. A: three sets of high salt plants. B: three sets of low salt plants. C: Mean value of the rates of uptake of the high salt and low salt sets and their difference (a crude index of the accumulation in the vacuoles of the low salt plants). D: The mean amounts of phosphate, taken up by the low salt and high salt sets, expressed in percentages of the total amount, taken up during the course of the experiment.

determined. A half-strength Hoagland solution was used. Fig. 10 shows the course of the uptake.

The course as a whole was the same for all sets (fig. 10: A and B). Initially there was an increase in the uptake in the light followed by a slight decrease. During the dark period the uptake fell rapidly. This fall decreased at the end of the dark period and changed into an increase during the second light period. It appears from the mean values of the high salt and low salt sets, that the uptake by the low salt plants was greatest (fig. 10: C). The initial increase was also clearest with this material. Both types of plants showed a large variability as to the uptake values. As a resuls of this the high salt set with the largest uptake is comparable with the low salt sets with the lowest uptake.

Experiment 6b. In this experiment low salt plants were exclusively used. Eight sets were grown during July and August 1948. They were six weeks old when used in the experiment. As a result of the favourable circumstances they were in excellent condition. Uptake rates were determined on Hoagland solutions of four different concentrations (12·5, 25·0, 37·5 and 50 %). At each concentration two sets were used. The course of the uptake is represented by fig. 11. The mean values of the two sets at each concentration is indicated.

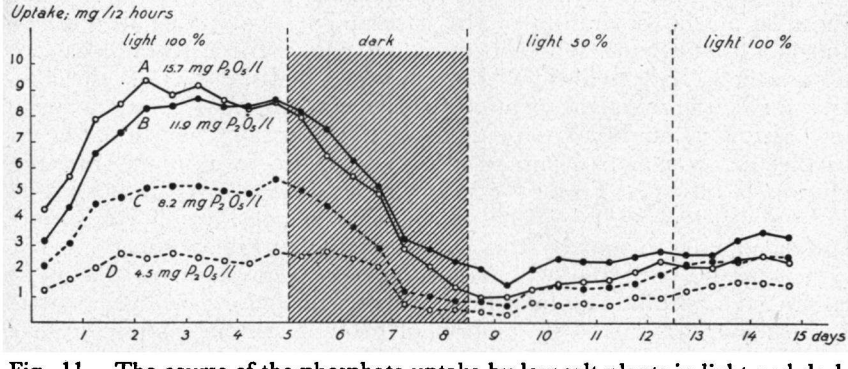

Fig. 11. The course of the phosphate uptake by low salt plants in light and darkness at different external phosphate concentrations.

The influence of the concentration is clear. The higher the concentration, the higher was the rate of uptake. The uptake also increased in this experiment. A constant level having been reached, the absorption rates were at increasing concentrations: 2·5, 5·3, 8·5 and 8·8 mg P_2O_5/12h. With the two sets at the highest concentration there was a small but definite decline in the uptake. But with the other sets a decline of the uptake, as occurred in the previous experiment, was out of the question. During the dark period the uptake of all sets, except for those standing on the lowest concentration, immediately decreased. With group A and B, this decline was larger than that with group C. With the fourth group the uptake continued

at the same rate for some days, but afterwards it also declined. At the end of the dark period all sets showed only a small uptake. Group B now absorbed rather more than group A.

If after this, the plants were again placed in light, the uptake rose only slowly after some retardation. This was due to the very low light intensity; two instead of four lamps being used. After four days the other two lamps were also switched on, but no clear influence could be observed. The uptake of group B remained the largest one, while the difference between A and C had disappeared.

Discussion

In order to explain the course of the uptake of the high salt plants it is obvious to think of the sugar supply, in view of its significance for the phosphate uptake. Besides, high salt roots are known to be in a low sugar condition. Hence, the cessation of photosynthesis in darkness may be expected to cause a fall of the phosphate absorption in a short time. For as much as the uptake depends on the consumption, the fixation by growth may be expected to be of primary importance. This growth is closely connected with the sugar supply. That is why it is dependent on the illumination. But it is conceivable that there are other effects of light on the growth and uptake.

The average uptake by low salt plants was larger than the uptake by high salt plants (fig. 10: C). Moreover, there was a marked increase at the beginning of the experiment. This must depend on the fact that the various processes, by which the rate of uptake is determined, had to be put into motion, because during the pretreatment, the whole absorption process was stopped. That this went on so slowly, doubtless depended on the low salt condition. As far as the uptake was determined also in this case by growth, this conclusion is obvious. From the previous section, growth appeared to be mainly depressed by phosphorus deficiency. Phosphorus supply enhanced growth and by this enhanced the nitrate absorption. The same can be expected with respect to the phosphate uptake. The enhanced phosphate uptake in its turn, will again promote growth. In this way a mutual promotion of growth and phosphate uptake could occur during the first days of the experiment.

At first sight the further course of the uptake by the low salt plants was of the same nature as it was with high salt plants. It is true that the fall in darkness was steeper and the rise in the light afterwards was smaller, so that there was less difference at the end of the experiment than there was at the start. Such solely quantitative differences also existed between the sets of one type of material. On closer examination however it appears that there are indeed vital differences.

This difference is demonstrated by comparing the average uptake rates of the high salt and low salt sets, if the absolute difference in uptake rates is neglected. For that purpose the average amounts of phosphate taken up during the twelve hours period, are expressed as percentages of the mean total amounts of phosphate, taken up by the two types of material. These percentages are plotted in figure 10: D.

It shows that the uptake by the high salt plants decreased in darkness and increased during the second period more intensively than did the uptake by low salt plants. Thus, the high salt plants were more sensitive to the alteration of light and darkness than were the low salt plants. As far as the decrease in darkness is concerned, this might be connected with the greater sugar storage of the low salt material. But, if this is so then it is difficult to see why afterwards, the increase in the uptake by low salt plants in light is so small. Rather, the different sensitivity points to a difference in the nature of the uptake in these two types of material. It is obvious to surmise that this is determined by the salt condition, as a result of which the uptake by the high salt plants is compounded in another way than is the uptake by the low salt plants. While uptake by high salt plants is largely dependent on the growth and accumulation in the vacuoles is of minor importance; with low salt plants, the accumulation in the vacuoles is also of importance. Both processes may depend on the illumination to a different extent. For with low salt plants sugar has been accumulated in the vacuoles giving at the same time an osmoregulation (HOAGLAND and BROYER 1936). Such an osmoregulating principle was also met with by DELEANO und ANDREESCO (1932) in leaves of *Salix*. They observed a loss of salts in senescent leaves. At the same time the concentration of monosaccharides was enlarged and thus, the osmotic concentration was kept at a fixed level. These data point to an intimate connection between ion accumulation in the vacuoles and the sugar stored in the vacuoles. If this assumption is correct, it may easily be seen why the accumulation with the low salt plants (and hence the total uptake by these plants) is far less sensitive to an alteration of light and darkness than it is with high salt plants.

To obtain an impression of the rate and the course of this accumulation in the vacuoles by the low salt plants, the difference between the absorption rates of low and high salt material might tentatively be taken. It should be realised that the uptake by the high salt plants was not solely based on the fixation by the growth. On the other hand, the growth of the low salt plants was not so vigorous as that of the high salt plants, at least not at the commencement of the experiment, owing to the unfavourable pretreatment. Hence, the accumulation rate of the low salt plants will certainly have been larger than the difference between the uptake of low salt and high salt plants. This difference, shown by fig. 10: C, must therefore be considered as a rough approximation.

As the figure shows, the accumulation in the vacuoles increased just a little at the start. It is difficult to judge the significance of this increase. Perhaps the true value of this increase was lower. Further, the rate remained at about a constant level during three days and then decreased gradually. There was not the slightest sign of any influence of light and darkness, so that the conclusion, low salt plants are less sensitive to light and darkness because their uptake is to a large extent due to the accumulation in the vacuoles, seems justified.

The diminishing of the difference in the uptake of low salt and high

salt plants indicates that the low salt sets came gradually into a high salt condition. On the grounds of the considerations just mentioned it could be expected that the smaller the change into a high salt condition, the smaller the sensitivity to a dark treatment. This was completely confirmed by the results of the second experiment. The higher the concentration of the solution that the plants were placed on, the higher the rate of uptake, and as a result of this the higher the velocity with which the plants attained a high salt condition. The effect on the course of uptake was in accordance with this, as is shown by fig. 11.

b. Experiments with high light intensity

With the experiments of 1949 incandescent lamps were used as a light source (fig. 1: B). As appeared from some of the experiments, under these conditions sugar supply to the roots was not a limiting factor, since the addition of glucose to the nutrient solution had no effect on the phosphate uptake (exp. 3*c*, *d*; 4*b*). The case that was used in the experiments concerning the influence of carbon dioxide-free air was removed, so that there was no danger during these experiments, which were prolonged for many days, that the growth of the plants was impeded. Moreover, it was now possible to measure the growth of the shoots and in this way to obtain an impression of the rate of the growth processes. The sets consisted of twelve plants and the measurements were made every twelve hours and hence very regular results could be obtained with these experiments.

In order to gain a further insight into the various factors influencing the uptake of high salt and low salt plants in light and darkness, some sets were harvested during these experiments and the phosphate contents determined.

Experiment 6c. The six sets of plants in this experiment were grown during the months May and June 1949. Conditions were not yet optimal. The plants were only small and this partly explains the low rate of uptake (fig. 12: B). During the first 2—3 days the uptake increased, it then continued at a constant rate. In darkness the uptake fell immediately, but soon attained a new constant level, which was nearly equal to the rate of uptake at the start of the experiment. This suggested that the uptake in light was a result of the promotion of growth. The course of the growth agreed with this. It increased during the first days, attained a constant level, fell in darkness and came virtually to a standstill. This development of the growth depends mainly on cell elongation which can continue even if the protoplasmic formation is stopped. Hence, the utilization of phosphate by the growth processes in these experiments will be stopped before the visible development comes to a standstill and also by this the absorption, as far as it was determined by this utilization.

At the end of the light period and of the sixth and seventh day, two sets were killed and analysed. If growth is checked in darkness, but uptake goes on at a reasonable rate, the phosphate content can be

expected to increase. As appears from table IV, the results are too variable to make it possible to conclude if this applies to this experiment.

Experiment 6d. The seven sets of this experiment were grown during the favourable summer months July and August 1949. The plants were more vigorous than those of the last experiment and the uptake was much larger. The light period lasted only two days, so the duration of the dark period could be extended to six days.

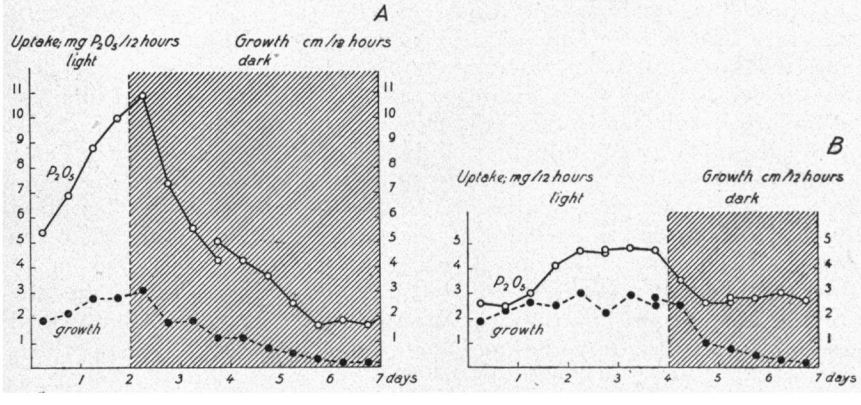

Fig. 12. The course of the phosphate uptake by low salt plants and the growth of their shoots in light (high intensity) and darkness. A: Sets of plants grown in midsummer. B: Sets of plants grown early in summer. The breaks of the curves are due to the removal of sets of plants.

As is shown by fig. 12: A, the uptake at once rose rapidly. The plants being placed in darkness this increase continued for some time and then the uptake diminished very gradually. After four days of darkness it had not yet stopped. On the contrary, it afterwards showed a constant, though low velocity. On the last day of the experiment the phosphate determinations were a little irregular; they have been omitted from the figure. The shoot development runs completely parallel to the uptake. The decrease in darkness was likewise very gradual. This decrease, however, continued until it at last became zero. The synthetic processes will be stopped earlier. Perhaps the breaking off in the decrease of the uptake on the sixth day was connected with this.

Two sets of plants were harvested at the end of the second, the fourth and the eighth day. There was an increment of the phosphate content in darkness (table IV). After the fourth day no significant alteration could be observed. The uptake by the sixth set was much lower than that by the other sets. The shoots of this set were excised on the second day. The uptake by the remaining root system fell rapidly at first, afterwards it rose again and though the course was irregular, the rate of the uptake at the end of the dark period was larger than that of the intact plants. Such an enhanced absorption rate by an excised root system was also observed in experiment 3*d*

(fig. 4). The correct explanation of this result is hard to give for the moment. At any rate it points to some interaction of shoot and root, important to the uptake.

Experiment 6e. High salt plants were used, grown during June 1949. There was a prosperous development. The most striking result of this experiment was the enormous increase in the uptake during the light period (fig. 13). Only after four days was a constant level attained. This behaviour, so different from that found with low light intensity can only be explained by the use of the high light intensity, and the large sugar production connected with it. This sugar production can enhance the accumulation in the vacuoles and the fixation of phosphorus by growth. However, neither of these two processes seems to give a sufficient explanation of the enormous increase of the uptake in light. Another possibility is an enhanced consumption by the formation of hexose phosphate. This hypothesis is very tempting in this case, for it can explain at the same time the rapid fall of the uptake in darkness. During the continued sugar consumption in darkness, bound phosphate will be liberated. As a result of this the uptake of inorganic phosphorus from the medium will be depressed and may even be stopped before the growth processes have come to a standstill.

The rhythm of the growth could be observed extremely well. This rhythm also occurred in the other experiments, though less clearly. The largest growth took place during the night in spite of the continuous illumination. The basis of this rhythm remains uncertain. It may be based on the cell divisions as well as on the cell elongations.

Analytical data were obtained at the beginning of the experiment,

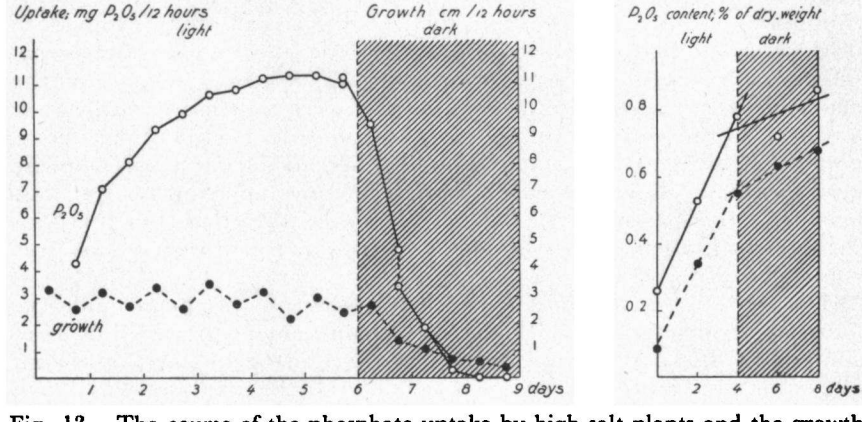

Fig. 13. The course of the phosphate uptake by high salt plants and the growth of their shoots in light (high intensity) and darkness. The breaks of the curves are due to the removal of sets of plants.

Fig. 14. The course of the phosphorus content of shoots and roots of low salt plants if they are allowed to take up phosphate from nutrient solutions, that are renewed twice a day.

at the end of the light period that lasted for six days, and after the first, second and third day in darkness. There was no sign of any alteration in the phosphorus content. But an obvious increment of fresh- and dry weight did occur. It was based mainly on the large growth of the shoots.

Experiment 6f. In order to obtain comparable data of low salt and high salt material five sets of each type were grown at the same time (June—July 1949). The sets were placed in the light during four days, and afterwards in darkness for four days. A high salt set and a low salt set were harvested at the start of the experiment and then every other day. When choosing the sets for analysis, some selection was applied in such a way that the sets at the start and at the end of the light and dark period were as equal as possible to look at. Thus the other sets, harvested at the fourth and sixth day, varied a little in development of the roots or shoots.

As a result of a failure of the photelometer the absorption values could not be determined exactly. For the sake of the analytical results the experiment was continued. Both kinds of material showed a large increase in the uptake during the first days. Only at the fourth day was a constant level attained. Afterwards, in darkness the uptake decreased gradually with both kinds of material by about the same extent. During the whole experiment the uptake by the high salt plants was larger than that of the low salt ones. This was a result of the low salt plants being less well developed owing to the salt deficiency.

The growth increased during the first days, then it remained at a constant level and diminished in darkness very slowly. During the light period all sets showed the day and night rhythm. The growth of the high salt plants was a little stronger than that of the low salt ones, throughout the experiment. The total picture looked very much like that obtained with the low salt plants of experiment 6*d*. (fig. 12: A). This involved that the high salt plants showed a behaviour differing from that during the last experiment. This may be connected with the conditions during the pretreatment. The growth was more rapid than with the high salt sets of the previous experiment, grown early in summer. This implied at the same time, that the plants did not attain an extremely high salt condition. This is also shown by the analytical results (table IV). On the other hand there was a good illumination allowing a sufficient sugar supply. It is not surprising that, owing to these various factors, the behaviour of the high salt plants was much like that of the low salt plants.

Discussion

A summarizing picture of the course of the uptake of low salt and high salt plants in light and darkness will be given in the general discussion. This discussion will be restricted to a few supplementary remarks concerning the analytical data. As had appeared in the description of the experiments, the value of the analytical results

TABLE IV

The fresh weight, dry weight, phosphorus content, total amount of phosphorus, and shoot- root ratio of the sets of plants, harvested at different moments in the experiments concerning the influence of light and darkness on the uptake of phosphate

Material	days	Shoot				Root				Shoot-root ratio	
		fresh weight g	dry weight g	P_2O_5 content %	total P_2O_5 mg	fresh weight g	dry weight g	P_2O_5 content %	total P_2O_5 g	fresh w.	dry w.
Low salt	4	44.8	4.00	0.77	30.8	36.0	2.66	0.89	23.7	1.2	1.5
Exp. 6*c*; fig. 12B		41.0	3.55	0.50	17.8	35.0	2.21	0.68	15.0	1.2	1.6
	6	60.0	5.16	0.67	34.6	47.3	2.84	1.02	29.0	1.2	1.8
		44.5	3.85	0.63	24.3	37.0	2.43	0.68	16.5	1.2	1.6
	7	59.0	4.83	0.80	38.6	47.0	2.69	1.08	29.1	1.3	1.8
		66.5	5.66	0.63	35.7	50.3	2.88	0.91	26.2	1.3	2.0
Low salt	2	77.0	8.14	0.47	38.3	50.5	3.24	0.52	16.8	1.5	2.5
Exp. 6*d*; fig. 12A		86.0	9.47	0.26	24.6	—	—	—	—	—	—
	4	75.0	7.36	0.65	48.6	42.0	2.63	0.65	17.1	1.8	2.8
		89.0	8.77	0.60	52.6	43.0	2.50	0.65	16.3	2.1	3.5
	8	—	9.19	0.60	55.1	—	2.78	0.82	22.8	—	3.3
		—	7.94	0.75	59.6	—	2.76	0.90	24.8	—	2.9
High salt	0	33.0	3.55	1.50	53.3	21.5	1.80	1.26	22.7	1.5	2.0
Exp. 6*e*; fig. 13	6	142.0	9.38	1.43	134.1	51.5	2.80	1.34	37.5	2.8	3.4
	7	236.0	17.17	1.04	178.6	78.0	4.40	1.13	49.7	3.0	3.9
	8	134.0	9.12	1.37	124.9	45.0	2.38	1.42	33.8	3.0	3.8
	9	206.0	13.34	1.08	144.1	64.0	3.19	1.33	42.4	3.2	4.2
High salt	0	154.0	14.28	0.80	114.2	60.0	3.91	0.88	34.4	2.6	3.7
Exp. 6*f*.	2	205.0	21.12	0.67	141.5	77.5	4.43	0.68	30.1	2.7	4.8
	4	216.0	21.30	0.73	155.5	62.5	2.90	1.02	29.6	3.4	7.3
	6	—	—	0.76	—	—	4.34	1.11	48.2	—	—
	8	150.0	12.90	1.17	150.9	58.0	2.50	1.62	40.5	2.6	5.2
Low salt	0	58.0	8.03	0.09	7.2	63.0	3.90	0.26	10.1	0.9	2.1
Exp. 6*f*.	2	69.5	7.81	0.28	21.9	46.0	3.02	0.49	16.2	1.5	2.6
	4	73.0	7.24	0.51	36.9	45.0	2.57	0.67	26.1	1.6	2.8
	6	—	7.85	0.59	46.3	—	3.24	0.79	24.4	—	2.4
	8	59.0	6.69	0.59	39.5	48.5	—	1.05	—	1.2	—

(table IV) is highly reduced by the large variability. This applies mainly to the fresh- and dry weight figures and hence to the absolute amounts of phosphate. Therefore only large differences find expression.

The fresh weight of the roots of the low salt plants was nearly the same as that of the shoots. With the high salt plants the fresh weight of the shoots was 2—3 times that of the roots. It is clear that the development of the shoots was highly depressed by the low salt treatment. The total amount of phosphate present in the low salt shoots equalled the amount present in the roots. With the high salt plants the ratio was also two or three. This means that during the pretreatment at least 2—3 times as much phosphorus was transferred to the shoots as was stored in the roots themselves. With a low salt treatment half of the phosphate absorbed was fixed in the root (WILLIAMS 1948). During the experiments the low salt plants were able to absorb large quantities of phosphate. In experiment 6*f* more than twice the amount that was accumulated in the roots themselves, was transferred to the shoots. In experiment 6*d* the ratio was even more favourable, as the shoots were relatively larger.

The most regular results are given in the figures of the phosphorus content. The values are expressed in percentages of total dry weight. The drawback however is, that alterations in phosphorus content may delude us. They may be the result of alterations in dry weight e.g. by the consumption or production of sugar. This disturbing factor will be taken into account as much as possible.

With high salt plants, no clear alterations in the phosphorus content could be observed. In experiment 6*f* the percentage appeared to increase in the darkness. This may be the result of a continued uptake and a simultaneous inhibition of the growth, so that the plants became saturated. This is in accordance with the course of uptake observed, which bore a close resemblance to that of the low salt plants. This pointed to their not being very high salt. Indeed, the phosphorus content was not as high as it was with the plants of experiment 6*e*. It is striking that the percentage rose mostly in the roots. With experiment 6*e* the plants were in an extremely high salt condition. No change of the phosphorus content occurred, nor could this be expected with material in which uptake was mainly determined by growth.

The low salt sets gave more obvious results. In spite of the large variability a rise in the phosphorus content could be observed during the first days of the experiment. This was due to the low content at the commencement of the experiment. In fig. 14 the data of various experiments have been summarized (fig. 12: A and B; exp. 6*f*). In two experiments the light period lasted for four days, in one experiment for only two days. In spite of this the data agrees rather well. There was an increase in the content during the first four days. Afterwards the increase was only slight and may be the result of the sugar consumption. This result supports the supposition that the uptake by low salt plants is to a large extent independent of the illumination and is determined by the salt and sugar condition (osmo-

regulation). The level finally attained almost coincided with that of the high salt plants of experiment 6*f* (0·69 resp. 0·76 %). This means that the plants though they did not develop to an extremely high salt condition, attained a reasonable salt level. With this material also, the phosphorus content of the roots was higher than that of the shoots.

The gradual diminishing of the differences between high salt and low salt plants also appeared from the course of the shoot-root ratio. The ratio values were variable. Two conclusions may be drawn. In the first place this ratio increased obviously with low salt plants during the experiments. This was connected with the very low value at the beginning of the experiments. In the second place, in spite of this increase, the favourable ratio of the high salt plants was not attained. The average value of this ratio with low salt plants after the fourth day of the experiments was: 1·4; with high salt plants: 2·8 (on dry weight basis: 2·4 resp. 4·1). Thus the ratio for high salt plants was twice that for the low salt ones. As far as the demand for salts is different between shoots and roots, this difference in shoot-root ratio must have had an influence on the uptake. The sensitivity to light is also very likely to be larger with plants with relatively large shoots. However, the large agreement in the course of uptake and growth between the high salt material and the low salt one of experiment 6*f* indicates that the salt condition is more significant than the shoot-root ratio.

IV. GENERAL DISCUSSION

Permeability, physico-chemical and biochemical principles

The first stage of the uptake by intact plants consists of an entering of the anions into the cytoplasm of the cells of the epidermis and perhaps into the cortical cells of the roots. Whether the anions can permeate into the cytoplasm easily is of primary importance. Therefore this problem needs to be discussed first.

In the experiments on the loss of salts it was found that amounts, comparable with those of the uptake, can be readily given off. This was conceived as an indication, that phosphate can permeate easily into and out of the cytoplasm of the root. The permeation was considered a passive process, in which special forms such as accelerated diffusion may play a part.

Meanwhile, Steinbach (1951) pointed out that this concept is an oversimplified one in the light of recent investigations. It is possible that ions are bound in the cell membrane to a specific substance. Then the complex is decomposed and the ion is liberated into the cytoplasm. On ground of analytical data for medium and cytoplasm in such a case the interference might be drawn erroneously, that there is an easy passive permeation. That which applies to the penetration of ions seems also to be applicable to their loss. Thus, Gale (1947) found that the loss of free lysine with *Streptococcus faecalis* depends on metabolism. A large loss of phosphate was obtained with *Chlorella*

pyrenoidosa and *Rhodospirillum rubrum* by KAMEN and SPIEGELMANN (1948). They could demonstrate that the leakage occurred mainly at the expense of the fraction extractable by trichloracetic acid. In this connection they point to the difficulty in determining the exact inorganic phosphorus content. They showed that by trichloracetic acid instable phosphorus compounds were broken down and extracted. This is important for the interpretation of the data. As KAMEN and SPIEGELMANN expounded, it seems that a choice must be made between two possibilities. Firstly it may be that the ion diffuses into the cell and is afterwards metabolised. Secondly, there may be some esterification on the surface of the cell followed by a breakdown as a result of which the ion is liberated into the cytoplasm. The experiments on the significance of the sugar supply and the preliminary results of the experiments on the phloridzin inhibition pointed to the second possibility. Moreover, discussing the results of the experiments over the relation of uptake and concentration, this second possibility proved to be a valuable hypothesis in the explanation of the "adsorptive" relation. It involved that the substance, to which phosphate is bound in first instance, is supposed to be present in a small amount. As a result of this the first stage may be the determining factor of the rate of the absorption process. STEINBACH also points to this possibility.

Meanwhile the two principles do not exclude one another. The possibility remains that ions also enter in a more physical way. This diffusion is generally assumed to be slow. This may be due to some outer membrane of specialized structure. It may also be the result of the presence of large indiffusible anions in the cytoplasm (ROBERTSON 1951; FREY-WYSSLING 1948). If the former is correct, the esterification process is necessary to transfer the anions through that outer membrane. It must be also localized in that membrane. Indeed this is generally accepted. This idea is supported by investigations e.g. those of Rothstein and collaborators (ROTHSTEIN and MEIER 1948), which showed that a lot of enzymes important for sugar metabolism are situated in the surface of yeast cells. Rothstein and Meier emphasize that the results need not mean a surface in a strict mathematical, two-dimensional sense.

However, if the resistance is supposed to be situated in the whole cytoplasm, the necessity to localize the active process in the surface, vanishes. Admittedly there may be some differentiation of the cytoplasm for this process. The phosphorylation processes, being so intimately connected to sugar metabolism, can be expected mainly to occur there, where the latter is dominant. With plant cells this applies (for example) to the outer layer of the protoplasm, that is engaged in cell wall formation (STREET and LOWE 1950). This also does not mean a localisation in a surface layer of molecular dimensions for, it is clearly shown by electronic microscopic pictures of young cell walls, that the cellulose elements are not only formed by the external membrane but by an outer layer of the cytoplasm of measurable thickness. The whole young cell wall is so to speak, soaked in

protoplasm (FREY-WYSSLING, MÜHLETHALER und WYCKOFF 1948; MÜHLETHALER 1950; ROELOFSEN and HOUWINK 1951). Phosphorylations are also coupled with the cyclophorase system that is to a large extent situated in the mitochondria.

One arrives at the conclusion that, if some esterification of phosphate is an essential condition for its uptake, this need not entail that the process goes on exclusively at the surface of the roots. Diffusion in its different aspects may play an additional part. Some differentiation between an outer and an inner layer of the cytoplasm seems likely. No doubt, uptake due only to diffusion processes is of minor importance. Change in the composition of the medium will cause the establishment of new Donnan equilibria. Initially some uptake or loss may occur as a result of this, as was found in the experiments on the loss of anions. In these experiments the plants were in a condition of low metabolic activity, large changes in the external concentrations being applied at the same time.

If these conclusions are correct, it means at the same time that a sharp distinction between entrance into the cytoplasm and a further transport in the cytoplasm or symplasm of the root cannot be made. The same principles may hold good for both. An important question remains viz. by what factor the direction of transport is determined.

The significance of metabolism, especially the significance of respiration to the anion uptake has been shown for years by Lundegårdh. The cytochrome-cytochrome oxydase system has been held responsible for the uptake in his well-known concept of the mechanism of anion transport. Small supplements have been added by BURSTRÖM (1951). ROBERTSON and his collaborators (WEEKS and ROBERTSON 1950) have attained results that support the principles of Lundegårdh's theory to a large extent. That the theory is not yet generally accepted in spite of this, might be ascribed to the following circumstances.

In the first place the cytochrome system take a central position in the whole of the energy supplying processes. Hence it is not surprising that the inhibition of this part of the respiration impeded the energy consuming process of anion absorption. A more complicated connection between uptake and respiration seems more likely (HOAGLAND 1948). This concept is supported by the data, that indicate the significance of phosphorylations (NANCE 1949; ROBERTSON, WILKINS and WEEKS 1951). It should however be admitted, that on the one hand, the quantitative relation between oxygen consumption and anion uptake is in good accordance with that which can be expected on grounds of Lundegårdh's view. On the other hand the effect of the phosphorylation might be due to their influence on the maintenance of enzymatic structures important for the ion uptake which are present in mitochondria (GREEN 1951). That in spite of this the relation of cytochrome and uptake is finally more indirect in its nature seems to be confirmed by the fact that anion uptake also occurs with cells and tissues which are lacking cytochromes. So

ÖSTERLIND found with *Scenedesmus quadricauda* that the respiration was not inhibited by cyanide, but the anion uptake was (ÖSTERLIND 1951).

A second point significant to the judgement of the cytochrome mechanism, is the specificity of the uptake. Ions are known to be absorbed in quantities, the ratio of which is quite different from that of the composition of the external solution. Moreover, the uptake of an anion proved to be independent of the uptake of other kinds of anions (ARISZ 1945; ALBERDA 1948; HUMPHRIES 1951). This could be affirmed by some experiments of the author not mentioned here: the uptake of phosphate was not affected by the presence or absence of other ions. Of course, secondary effects may occur. This was clearly demonstrated by the experiments on the influence of the utilization on the uptake of nitrate by phosphorus deficient plants.

This independent behaviour of the uptake of various anions can with difficulty be squared with the unspecific cytochrome mechanism. It requires at least some supplementary mechanism, that is adjusted to the nature of the anion. This might consist of binding it to some carrier. This formation may be connected with metabolism (STEINBACH 1951) so that the cytochrome system may be concerned with it. Afterwards, the anion, once it has entered into the cytoplasm, might be transported by the cytochrome system as it is assumed to be. The question whether this applies, is closely connected with the question as to the form in which phosphate is transported. ARISZ (1952) indicated the resemblance in the transport of organic substances like amino acids and inorganic ions. In particular, with regard to phosphate it seems very likely, that to a larger extent it is transported in a bound rather than in a free state. Thus, phosphate transport may be compared with that of organic substances. The same principles: mass flow, diffusion, protoplasmic streaming may be valid.

The problem remains concerning the polarity of the transport. Lundegårdh assumed two factors. In the first place the direction might be determined by an oxygen gradient. He does not deem it to be an important factor, because large variations in the oxygen percentage of the medium are possible without any effect on the uptake. Then the direction must be due to some polarity of the structure of the cytochrome system. Thus the localisation of this cytochrome system is important. During the last years this system has proved to be bound to the mitochondria (GREEN 1951; MILLERD e.a. 1951). It is difficult to say how the polarity of an uptake, that is connected with this system, given its localisation, can be explained.

In the opinion of the author such a polarity of the transporting mechanism is not a necessary requirement. There is a continuous consumption of ions, as they are fixed by synthetic processes, and secreted into the vacuoles and vessels of the root. What is required is some mechanism that levels concentration gradients in the symplasm of the roots. This might be a cytochrome mechanism or the protoplasmic streaming (also sensitive to HCN) by which ions, bound to protoplasmic particles (ARISZ 1945) or mitochondria (ROBERTSON 1950) or not, are transported in every direction. Such an undirected

transport also explains the loss of ions if the utilization of the plants stops and dissimilatory processes become dominant.

Some remarks should be made concerning the possible role of the epidermis and cortex. According to BURSTRÖM the oxydative stage of the uptake would be situated in the epidermis. This concept is supported by the experiments of SANDSTRÖM (1950), who treated the roots with di-amylic acetic acid by which the cells of the epidermis are ruptured. The uptake by plants in which the epidermis has been peeled off in this way looks like a passive absorption of the ions owing to the transpiration stream. Particularly, the specificity of the anion uptake disappears. BROYER and HOAGLAND (1943) found that damage to the roots also results in a more passive uptake. Further examination of the effects of di-amyl acetic acid is desirable.

As distinct to this concept of BURSTRÖM many investigators assume the cortex as a whole to be in direct contact with the medium. This may be due to a great passive permeability or to the transpiration stream by which the ions can be transported passively at least through the cell walls of the cortex.

Summarizing it can be stated that biochemical factors are decisive as to the phosphate uptake. In view of the fact that inorganic phosphate is bound very rapidly (gel-P; Green 1951) this is not surprising. This renders it at the same time somewhat uncertain as to whether the mechanism of the phosphate uptake may be used as a model for the uptake of other anions (STEINBACH 1951). During the investigations discussed here, the need was felt more and more for studying also the uptake of another anion, that is linked up with metabolism to a lower extent. Unfortunately, the maize plant used, proved to absorb only a very small amount of chloride if any at all. However, the more significant metabolism is for the uptake of anions, the more it will be necessary to take into account the specific features of the anion in question. It is surely possible that the quantitative significance of the various absorption and transport principles depends on the nature of the anion.

PHYSIOLOGICAL FACTORS

The factors determinative for the uptake by the roots have been mentioned previously: the accumulation in the vacuoles, the secretion into the vessels, the fixation by synthetic processes (growth) and the supply of salts from the shoot.

The fact that the concentrations in the vacuoles of algae may be many times that of the external solution, was the starting point of the modern research into the salt accumulation. Afterwards the close connection between the absorption of ions and metabolism particularly with excised root systems and slices of storage tissue was stressed (STEWARD 1935; HOAGLAND and BROYER 1936; HOAGLAND 1948). Generally no sharp distinction was made between the absorption from the medium and the accumulation in the vacuoles. The active absorption mechanism was thought to be situated in the surface of

the absorbing cells. Such a distinction between the uptake of anions into the cytoplasm and their secretion from the cytoplasm into the vacuole was made by ARISZ in 1948. With *Vallisneria* leaves he observed an accumulation of ions in the vacuoles in light. In darkness there was no uptake. If a part of the leaf, though not being in contact with the medium was illuminated, it showed accumulation in the vacuoles. The non-illuminated part of the leaf that was in contact with the medium had to absorb the substances. Then they were translocated to the illuminated part and there they were secreted into the vacuoles (vacuole-secretion theory: Arisz 1948). Thus we are fully justified in using the term vacuole-secretion, by which the similarity with the secretion to the vessels is stressed. In agreement with the usual terminology, accumulation has been used throughout this article. To avoid misunderstanding distinction has been made between total absorption and this accumulation in the vacuoles.

The significance of the accumulation in the vacuoles for the total absorption depends on the salt condition. With low salt plants it is to a large extent determinative in fixing the rate of absorption. As a rule the uptake by low salt plants will be larger than that of the high salt ones. On the other hand, owing to the low salt treatment the development of the plants is checked, mainly by the phosphorus deficiency. By this, in spite of the low salt condition, the rate of uptake may be lower than that of the high salt plants. The different composition of the uptake appears from the behaviour in darkness. The uptake by the low salt plants is less sensitive to a dark treatment. The accumulation in the vacuoles even continues undisturbed. But this accumulation decreases gradually during the course of the experiment as the material is attaining a higher salt condition. Along with this, the sensitivity of the total uptake increases. Low salt plants have a large amount of available sugar in their roots (HOAGLAND and BROYER 1936; ALBERDA 1948). It appears that this sugar is mainly of benefit to the accumulation. The result is that sugar is replaced by salt just as during the pretreatment sugar was stored instead of salt. This results in some sort of osmoregulation.

Another process by which the uptake is determined is the fixation of ions by synthetic processes. That the uptake can be enhanced or limited by growth seems very obvious on a priori grounds. But, if it is true, that even the very first stage of the uptake is connected with metabolism, such a direct regulating influence of the growth is far from being self-evident. Both processes, uptake and growth, may be based on the same metabolic conditions. A direct causal analysis is necessary, as was made in these experiments concerning the nitrate absorption. Nitrate utilization was inhibited by phosphorus deficiency, allowing a regulation of growth by phosphate supply.

The extent to which uptake is determined by growth processes, depends on a number of factors. Large differences may be found between nitrate, phosphate, sulphate and chloride as they are utilized by the plant to a different degree. Secondly, the external concentration is significant. With low concentrations the first stage of the absorption

process may be limiting. This was observed in the experiments on the relation of uptake and external concentration. This process may also be limiting at higher concentrations viz. if the substance to which the anion is assumed to be bound, is present to a limited amount. If this does not apply, the utilization by the plants may impede a further increase in the uptake for rising external concentrations. This effect occurs only occasionally with low salt plants. On the other hand, with high salt plants it was obligatory. This is intelligible since the salt condition of the latter implies that the total consumption is mainly due to growth. As appeared from the experiments on the influence of light and darkness and on the effect of carbon dioxide-free air on the uptake, this growth, and through this the uptake, is sensitive to the sugar supply.

As well as being influenced by the salt and sugar condition of the plants, growth is influenced by a number of other factors, that may be partly hormonic in nature. The result is, that the growth of the root, and hence the uptake as far as it is influenced by this growth, also becomes dependent on factors and processes of medium and shoot.

The most specific activity of the root consists of the secretion of salts into the xylem vessels. With a reasonable salt supply the amount translocated to the shoot can be the larger part of the uptake, as is proved by considerable data concerning the salt content of roots and shoots. It could be deduced from the data of the plants used in these experiments, that with low salt material, equal amounts were present in the shoots and the roots; with high salt material the amount transferred to the shoots was 2—3 times the amount stored in the roots. A possible loss from the shoots to the roots is not taken into account. Perhaps the ratios were even more favourable.

Many investigators have employed themselves in the study of this process, mainly in connection with the examination of the exudation process. For this latter process the transport of water, i.e. the rate of exudation, is also important. As far as the salt secretion is concerned, it was found, as with the accumulation in the vacuoles, that the concentration of ions in the exudate may be higher than it is in the medium (LAINE 1934; HOAGLAND and BROYER 1942; LUNDEGÅRDH 1945). This requires an active process. The problem is, whether this process is required to bring the ions from the medium into the symplasm of the root or to transport them in the symplasm, or to secrete them from the symplasm into the vessels or some combination of these processes.

As was mentioned above, ARISZ made a distinction between the penetration into the cytoplasm, a further translocation and a secretion into the vacuoles. Thus, there exists a stream of ions into the vacuoles and a stream of ions circumventing the vacuoles, into the vessels (ARISZ 1945). This concept is supported by the experiments of BROYER (1950). He found that if roots, having been able to absorb much bromide, were placed in a solution with radioactive bromide, the exudate is very rich in active bromide. It indicates that the bromide stored in vacuoles is retained and is hardly transferred at all.

As to the mechanism of the secretion into the vessels, equally little is known, as is the case with the accumulation in the vacuoles. It is an attractive working hypothesis to consider them together as processes related to secretion by the salt glands of salt plants (ARISZ and HEYKENS, unpublished results). As early as 1938, CRAFTS and BROYER gave their hypothesis concerning the transport of ions from the medium into the vessels. On ground of anatomical features of the cortex and the stele of the roots the oxygen supply is assumed to be more favourable to the cortex than to the stele. That is why the cortical cells are able to accumulate large amounts of ions that are transported to the stele. Owing to the imperfect oxygen supply the cells surrounding the xylem vessels cannot maintain a high "accumulation level" (WIERSUM 1947) so that a leakage of the ions into the vessels occurs. This line of thought can easily be applied to the special case of phosphate transport. Cells with a normal active metabolism prove to dispose of large quantities of instably bound phosphate (KAMEN and SPIEGELMANN 1948; GREEN 1951) that is easily liberated so that it can be considered as if it was an inorganic phosphate. Apart from this, in experiments over the loss of phosphate, it was shown that under conditions of low metabolic activity, phosphate is readily given off. So such a leakage might easily occur as a "normal" phenomenon within the stele. The resistance to a diffusion of free anions in the symplasm might prevent the ions from being transported to cortex and medium.

It is more difficult to judge how far the hypothesis is correct as to the transport of the other ions. It also remains uncertain whether the oxygen supply of the stele is insufficient as it is obvious to assume this on ground of anatomical data. It may even be doubted as to whether a low oxygen tension is an essential condition for the loss of ions. For, with the salt glands on the leaves of halophytes a secretion also occurs. An insufficient oxygen supply seems to be very unlikely. LUNDEGÅRDH (1950) arrives at the conclusion on ground of his results with inhibitors, that the uptake into the root and the secretion into the vessels are different processes. For the uptake into and the further transport in the symplasm the anion respiration is necessary (up-hill reaction). The loss of ions into the vessels (down-hill reaction) is connected with glycolytic metabolism. Thus it is possible that there are different enzyme systems and hence differences in metabolism in the secreting cells and the absorbing cells of the cortex.

The exudation phenomenon is also studied in order to obtain some insight into the process of water intake (KRAMER 1949). The main problem is whether this transport is an active or a passive one. The question, as to whether the difference in osmotic value between exudate and medium is sufficient to explain the rate of exudation, has been the object of some recent investigations (VAN OVERBEEK 1942; EATON 1943; VAN NIE, HELDER and ARISZ 1950). The authors last mentioned arrived at the conclusion that the osmotic regulation is an important factor, at least in tomato plants. In this there is difference of opinion with Lundegårdh, who attaches less and less

value in his latest articles to the osmotic value of the exudation sap. This difference in valuation of the osmotic component of the exudation does not involve a complete contradiction between the concepts of these authors and Lundegårdh, as has been claimed by BURSTRÖM (1951). As appears from the more elaborate article of ARISZ, HELDER and VAN NIE (1951), the importance of the salt secretion for the exudation is fully recognized. There is an intimate connection between salt secretion and water transport and a mutual influencing.

As for accumulation and growth, the transport of ions and water from the medium into the vessels depends on the sugar supply (WENT 1944). The importance of the salt condition was mentioned. It points to a competition between the various processes: fixation by growth, accumulation in the vacuoles and secretion into the vessels. With a low salt condition and a low external salt concentration, a large part of the salt absorbed is retained by the roots themselves. An insufficient salt supply to the shoot will influence the activities of the roots which may lead to a better salt supply from the roots to the shoots. How far there is a direct regulating influence on the secretion into the vessels of the root is unknown.

Finally, the uptake is influenced by the loss of ions from the shoot. This loss in its turn is connected with the entire salt metabolism of the shoots. Perhaps, the processes of uptake by shoots are comparable to those by roots. If this applies, it means that one has to distinguish between entry into the cytoplasm, transport in the cytoplasm, fixation by growth processes, accumulation in the vacuoles and loss to the sieve tubes. If the uptake has been split up in such a way it will be clear that there might also be some differences. The medium of the shoot tissue is formed by the content of the xylem vessels. By means of the transpiration and exudation stream new amounts of ions are steadily supplied. During the experiments, mentioned here, the transpiration was only slight owing to the humid conditions. Guttation of the leaves was very often observed. It is not very likely that under these conditions a selective absorption occurs, for it would cause a concentration of the ions to form in the vessels. This renders a more or less passive penetration of the ions into the protoplasm more likely. The situation might be compared with roots deprived of their epidermis (BURSTRÖM 1951; SANDSTRÖM 1950).

With a limited consumption of the ions, absorbed in this way, a surplus arises that is transported back to the root by the phloem vessels (BIDDULPH 1951). The final result is a circulation of ions within the plant. For a further discussion of the principles of translocation of substances in the sieve tubes and the significance of such a circulation, one may be referred to a recent review (ARISZ 1952). Whereas the salt- and sugar condition may play an equal part in these various processes in the shoot as they do in the root, the translocation to the roots may in particular be linked up with the translocation of the sugar in the sieve tubes.

THE UPTAKE BY THE INTACT PLANT

An analysis of the absorption behaviour by intact plants was the very beginning of these experiments. In explaining the course observed, the several factors and processes mentioned up till now, will have to be taken into account. As there were some hypothetical elements involved in the principles mentioned, if we have to take into account the interaction of the different processes, the hypothetical element becomes greater. A consideration of the physiology of the salt relations in the intact plant cannot be anything but speculative in nature. The discussion will therefore, be restricted to one concrete case viz. the decrease in the salt absorption when the plants are placed in darkness.

It was obvious from the experiments concerning the influence of a dark treatment and the withdrawal of carbon dioxide, that the effect of the light factor is mainly an indirect one (photosynthesis). A direct influence on the accumulation into the vacuoles was observed by ARISZ (1947). Luttkus und Bötticher (1939) assumed potassium in the leaves of maize plants to be bound to an unknown protoplasmic substance when the leaves are in the light. VAN DER BURG (1952) showed that the cessation of the potassium uptake in darkness was due to the cessation of photosynthesis. If the sugar supply stops, all energy consuming processes e.g. growth or accumulation in the vacuoles, diminish. The extent to which this happens depends on the amount of sugar avaiable, which is in its turn correlated with the salt condition. Moreover, the various processes are sensitive to the sugar supply to a variable degree. At any rate the final result is a decreased consumption of salt by the shoot. If the salt supply from the root remains the same, a surplus originates in the shoot, which together with the ions continuously liberated in the aging parts is transported back to the root. This loss of salts by the shoot is highest for high salt plants, because firstly the supply from the root is large, secondly the consumption is mainly determined by growth that is very sensitive to a dark treatment and finally the loss by the older parts is large. These salts coming from the shoots, can be expected to compete immediately with the ions of the medium. At the same time the decreased sugar supply will enhance the depressing effect on the absorption from the medium, because it can diminish the uptake from the medium into the symplasm of the root. As to the phosphate absorption, where this first stage depends on an esterification, the connection of this first stage with the sugar metabolism is very likely. To what extent it is important to other ions is difficult to say. The transport in the cytoplasm, the growth processes, the accumulation in the vacuoles and the secretion into the vessels also depend on the sugar supply. The salt and sugar condition is important again in this connection. The final result is that the uptake from the medium, in particular by high salt plants, is more sensitive to the alterations of light and darkness than that of low salt plants.

Next to the loss of salts and the diminished sugar supply from the shoots, other special factors may play a part. Hormonic influences

on the growth of the shoots as well as on the roots, and as a result of this hormonic influences on the uptake are possible. It is quite unknown how far such influences exist e.g. on the secretion into the vessels, and in what way ions stored in the vacuoles are liberated and made available to the shoots also remains an unsolved problem.

As was admitted this scheme is speculative It is given in order to obtain a preliminary review of the salt relations of the intact maize plants. At the same time it may serve not only to show how complex the uptake by intact plants may be, but also to illustrate that, except for the absorption problem in its strict sense, there remain many typical physiological problems concerning the salt uptake of higher plants.

V. SUMMARY

In all experiments sets of intact maize plants were used, high salt as well as low salt ones. As a rule the phosphate uptake was determined and occasionally the nitrate uptake. In some experiments the growth was determined by tracing the development of the shoots.

The starting point was the examination of the course of the uptake of phosphate and the growth in light and darkness. Initially the uptake increases in light, in particular with low salt material. But with an intensive illumination the uptake by high salt material also increases. After some days a constant level is attained. In darkness the uptake decreases. The extent to which this occurs with low salt material depends on the degree in which the plants have been turned into a high salt condition. This can be regulated experimentally by the use of various concentrations.

The growth of the shoot increases mostly only a little during the first days of an experiment. Afterwards it remains at a constant level but shows a typical day and night rhythm in spite of constant conditions. In darkness the growth drops. Generally uptake and growth progress parallel to each other, but there are exceptions.

It appeared from analysis of the plants, that a large amount of the phosphate absorbed is transferred to the shoot. With an extremely low salt condition the amounts present in the shoot and root are almost equal. If the plants are standing on a nutrient solution, a large part is transferred to the shoot.

In explanation of the behaviour observed, the uptake from the external solution was assumed to depend on a number of physiological factors: fixation by growth processes in root and shoot; accumulation in the vacuoles; secretion into the vessels; loss from the shoots. The importance of accumulation and secretion is obvious. The effect of the loss from the shoot (as far as it can be studied from a single experiment) could not be obtained.

On the other hand growth proved to be of primary importance. In order to show this, high nitrate, low phosphate plants were grown. By lack of phosphorus, growth was checked. Some nitrate absorption was possible but it soon came to a standstill, the plants coming in

a nitrate saturated condition as a result of the daily renewal of the solutions. By phosphate supply the synthetic processes could get started. This leads on the one hand to a renewed nitrate absorption, on the other hand to the promotion of the visible growth.

All processes mentioned are connected with metabolism. That is why sugar supply can be expected to have an influence. As a matter of fact carbon dioxide-free air proved to depress the uptake in the same way as does a dark treatment. The uptake could also be enhanced by the addition of glucose to the solution. At intensive illumination it does not occur. With low salt material a prolonged dark treatment may be necessary before a clear effect of the glucose addition is obtained. Hence it is not surprising that the uptake by low salt material is less sensitive to the changing of light and darkness, than is high salt material. Perhaps, this is mainly due to the accumulation in the vacuole being less sensitive to sugar supply, since much sugar has been stored in the vacuoles (osmoregulation).

Whether there is an effect of glucose addition or not, also depends on the external phosphate concentration. It was found, in accordance with the common concepts that the relation between concentration and uptake can be represented by an adsorption isotherm (Freundlich- or Langmuir curve). The well-known "saturation phenomenon" was also obtained. If the absorption activity of the plants increases, the uptake at a lower concentration is more enhanced than at a higher one. If glucose has an enhancing effect, then the higher the concentration the larger the effect.

To explain the regularities observed, the uptake of phosphate was assumed to start by becoming bound to some specific substance which is present in a limited amount. The preliminary data concerning the inhibition of the uptake by phloridzin also points to such a phosphorylation. Following on this initial binding the phosphate is translocated inwardly the symplasm of the roots. This translocation may be influenced by glucose. Langmuir's formula proves to be of primary importance in this concept. It renders a calculation of the theoretical "saturation" value possible. In nearly all cases where a maximum rate of the uptake was found, this appeared to be lower than this theoretical "saturation" value. This must be connected to a limiting influence of the consumption.

The significance of the metabolism for the phosphate uptake is obvious. However, experiments on the rapid loss of phosphate depending on the concentration of the medium, point to a large passive permeability. The significance of active and passive processes on the anion uptake was discussed in detail. It is emphasized that in general, the various principles, factors and processes important to the uptake, do not exclude one another. Nevertheless their quantitative meaning will depend on the certain known conditions e.g. low salt condition of the plants. Next to this the nature of the anion which is absorbed, particularly the extent to which it is taken up by the cytoplasm or the extent to which it is secreted into the vacuoles, is very important.

LITERATURE CITED

ACHROMEIKO, A. J. (1936). Uber die Ausscheidung mineralischer Stoffe durch Pflanzenwurzeln. Zeitschr. f. Pflanzenernähr., Düng. u. Bodenk. *42*, 156—86.

ALBERDA, TH. (1948). The influence of some external factors on growth and phosphate uptake of maize plants of different salt conditions. Rec. trav. bot. néerl. *41*, 541—601.

ANDEL, O. M. VAN, W. H. ARISZ, and R. J. HELDER (1950). Influence of light and sugar on growth and salt intake by maize. Proc. Kon. Akad. Wet. *53*, 159—71.

ARISZ, W. H. (1945). Contribution to a theory on the absorption of salts by the plant and their transport in parenchymatous tissue. Proc. Kon. Akad. Wet. *48*, 420—46.

ARISZ, W. H. (1947). Uptake and transport of chlorine by parenchymatic tissue of leaves of *Vallisneria spiralis*. II. Analysis of the transport of chlorine. Ibid. *50*, 1235—45.

ARISZ, W. H. (1948). Uptake and transport of chlorine by parenchymatic tissue of leaves of *Vallisneria spiralis*. III. Discussion of the transport and the uptake. Vacuole secretion theory. Ibid. *51*, 25—33.

ARISZ, W. H. (1952). Translocation of organic compounds. Ann. Rev. of Plant Physiol. *3*, 109—30.

ARISZ, W. H., R. J. HELDER and R. VAN NIE (1951). Analysis of the exudation process in tomato plants. J. exp. Bot. *2*, 257—97.

ASANA, R. D. (1949). The absorption of nitrogen by the sugar-cane plant at different stages of growth. Ann. of Bot. N. S. *13*, 237—40.

BIDDULPH, O. (1951). The translocation of minerals in plants. Mineral Nutrition of Plants. Univ. of Wisconsin Press. 261—75.

BOOY, H. L. and H. P. WOLVEKAMP (1944). Catenary processes, master reactions and limiting factors. Biblioth. biotheor. Ser. D, *1*, 145—224.

BRANDT, K. (1942). Ueber Phosphatausscheidung und Permeabilität der getrockneten Bäckerhefe. Biochem. Zeitschr. *312*, 89—99.

BRIGGS, G. E. and R. N. ROBERTSON (1948). Diffusion and absorption in discs of plant tissue. New Phytol. *47*, 265—83.

BROOKS, S. C. and M. M. BROOKS (1941). The permeability of living cells. Protoplasma Monogr. Borntraeger, Berlin.

BROYER, T. C. (1950). Further observations on the absorption and translocation of inorganic solutes using radioactive isotopes with plants. Plant Physiol. *25*, 367—77.

BROYER, T. C. (1951). The nature of the process of inorganic solute accumulation in the roots. Mineral nutrition of plants. Univ. of Wisconsin Press, 187—249.

BROYER, T. C. and D. R. HOAGLAND (1943). Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Amer. J. Bot. *30*, 261—73.

BULL, H. B. (1951). Physical biochemistry. John Wiley & sons, Inc. New York. Chapman & Hall Lim. London.

BURD, J. S. (1919). Rate of absorption of soil constituents at successive stages of plant growth. J. agric. Res. *18*, 51—72.

BURG, A. H. VAN DER (1952). Influence of light on the absorption of potassium by maize plants in carbondioxide-free air. Proc. Kon. Akad. Wet. ser. *C 55*

BURSTRÖM, H. (1947). A preliminary study on mineral nutrition and cell elongation of roots. Kungl. fysiogr. Sällsk. Lund Förhandl. *17*.

BURSTRÖM, H. (1948). Mineral nutrition of plants Ann. Rev. Biochem. *17*, 579—99.

BURSTRÖM, H. (1949). Studies on growth and metabolism of roots. I. The action of n-diamylacetic acid on root elongation. Physiol. Plant. *2*, 197—209.

BURSTRÖM, H. (1951). Mineralstofwechsel. Fortschr. d. Bot. *13*, 250—68

BURSTRÖM, H. (1951). The mechanism of ions absorption. Mineral nutrition by plants. Univ. of Wisconsin Press, 251—260.

CRAFTS, A. S. and T. C. BROYER (1938). Migration of salts and water into xylem of the roots of higher plants. Amer. J. Bot. *25*, 529—35.

DAVIES, R. E. and A. G. OGSTON (1950). The mechanism of acid secretion by gastric mucosa and by other tissues. Bioch. Journ. *46*, 324—33.

DELEANO, N. T. und M. J. ANDREESCO, (1932). Beiträge zum Studium der Rolle

und Wirkungsweise der Mineral- und organischen Stoffe im Pflanzenleben. I. Der quantitative Stoffwechsel der Mineral- und organischen Substanzen in der Salix fragilis-blättern während ihre Entwicklung. Beitr. z. Biol. d. Pflanzen *19*, 249—84.

EATON, SCOTT V. (1949). Effects of phosphorus deficiency on growth and metabolism of sunflower. Bot. Gaz. *110*, 449—64.

EATON, SCOTT V. (1950). Effects of phosphorus deficiency on growth and metabolism of Soy bean. Bot. Gaz. *111* 426—36.

EATON. F. M. (1943). The osmotic and vitalistic interpretations of exudation. Amer. J. Bot. *30*, 663—74.

FRANCO, C. M. and W. E. LOOMIS (1947). The absorption of phosphorus and iron from nutrient solutions. Plant Physiol. *22*, 627—34.

FREELAND R. O. (1937). Effect of transpiration upon the absorption of mineral salts. Amer. J. Bot. *24*, 373—74.

FREY-WYSSLING, A. (1948). Submicroscopic morphology of protoplasm and its derivatives. Elsevier Publ. Comp. Inc. Amsterdam.

FREY-WYSSLING, A., A. K. MÜHLETHALER und R. W. G. WYCKOFF (1948). Microfibrillenbau der pflanzlichen Zellwände. Experientia *4*, 475—76.

GALE, E. F. (1947). The assimilation of amino acids by Bacteria. 1. The passage of certain amino acids across the cell wall and their concentration in the internal environment of Streptococcus faecalis. J. gen. Microbiol. *1*, 53—76.

GREEN, D. E. (1951). The cyclophorase complex of enzymes. Biol. Rev. *26*, 410—55.

GREGORY, F. G. (1937). Mineral nutrition of plants. Ann. Rev. Biochem. *46*, 557—78.

HAUROWITZ, F. (1948). Fortschritte der Biochemie 1938—47. C. Karger Basel, New York.

HELDER, R. J. (1951). Growth as a determining factor for the intake of anions by maize plants. Proc. Kon. Akad. Wet. ser. C *54*, 275—86.

HINSHELWOOD, C. N. (1946). Chemical kinetics of the bacterial cell. Oxford Univ. Press, London.

HOAGLAND, D. R. (1948). Lectures on the inorganic nutrition of plants. Chronica Bot. Comp. Sec. print. Waltham Mass.

HOAGLAND, D. R., and T. C. BROYER (1936). General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. *11*, 471—507.

HOAGLAND, D. R. and T. C. BROYER (1942). Accumulation of salt and permeability in plant cells. J. gen. Physiol. *25*, 865—80.

HÖBER, R. (1945). Physical chemistry of cells and tissues. J. & A. Churchill Ltd. London.

HONERT, T. H. VAN DEN (1933). The phosphate absorption by sugar cane. Leiden.

HONERT, T. H. VAN DEN (1936). Beperkende factoren bij de phosphaat opname (with English translation). Leiden.

HORNBERGER, R. (1882). Chemische Untersuchungen über das Wachstum der Maispflanze. Landw. Jahrb. *11*, 359—523.

HUMPHRIES, E. C. (1950). The absorption of ions by excised root systems. I. Apparatus and preliminary experiments. J. exp. Bot. *1*, 282—300.

HUMPHRIES, E. C. (1951). The absorption of ions by excised root systems. II. Observations on roots of barley grown in solutions deficient in phosphorus, nitrogen or potassium. J. exp. Bot. *2*, 344—79.

JACOBSON L. and R. OVERSTREET (1947). A study of the mechanism of ion absorption by plant roots using radioactive elements. Amer. J. Bot. *34*, 415—20.

JACOBSON L., R. OVERSTREET, H. M. KING and R. HANDLEY (1950). A study of potassium absorption by barley roots. Plant. Physiol. *25*, 639—47.

JENNIE, H. and R. OVERSTREET (1939). Surface migration of ions and contact exchange. J. Phys. Chem. *43*, 1185—96.

KAMEN, M. D. and S. SPIEGELMAN, (1948). Studies on the phosphate metabolism of some unicellular organisms. Cold Spring Harbor Symposia on quantitative Biology *13*, 151—63.

KNOWLES, F. and J. E. WATKIN (1931). The assimilation and translocation of plant nutrients in wheat during growth. J. Agr. Sci. *21*, 612—37.

KRAMER, P. J. (1949). Plant and soil water relationships. McGraw–Hill Book Co. Inc. New York.
LAINE, T. (1934). On the absorption of electrolytes by the cut roots of plants and the chemistry of plant exudation sap. Acta Bot. Fennica. *16*, 1—64.
LOOMIS, W. E. and CH. A. SHULL (1937). Methods in plant physiology. McGraw–Hill Book Comp. Inc. New York, London.
LUNDEGÅRDH, H. (1945). Absorption, transport and exudation of inorganic ions by the roots. Ark. f. Bot. *32 A* (12), 1—139.
LUNDEGÅRDH, H. (1947). Mineral nutrition of plants. Ann. Rev. Bioch. *16*. 503—28.
LUNDEGÅRDH, H. (1950). The translocation of salts and water through wheat roots. Physiol. Plantarum *3*, 103—51.
LUTTKUS, L. und R. BÖTTICHER (1939). Über die Ausscheidung von Aschenstoffen durch die Wurzeln. Planta *29*, 325—40.
MACHLIS, L. (1944). The respiratory gradient in barley roots. Amer. J. Bot. *31*, 281—82.
MASON, T. G. and E. J. MASKELL (1931). Further studies on transport in the cotton plant. I. Preliminary observations on the transport of phosphorus, potassium and calcium. Ann. Bot. *45*, 125—73.
MASON, T. G. and E. PHILLIS (1945). The effect of ringing and of transpiration on mineral uptake: A reply to criticism. Ann. Bot. N.S. *9*, 345—51.
MAXIMOW, N. H. (1938). Plant Physiology. McGraw–Hill Book Co. London.
MCINTYRE, G. A. and R. F. WILLIAMS (1949). Improving the accuracy of growth indices by the use of ratings. Austr. J. sci. Res. ser. B *2*, 319—45.
MILLERD, A., J. BONNER, B. AXELROD, and R. BANDURSKI (1951). Oxidative and phosphorylatic activity of plant mitochondria. Proc. Nat. Acad. Sci. *37*, 855—62.
MOORE, R. F. (1949). Downward translocation of phosphorus in separated maize roots. Amer. J. Bot. *36*, 166—69.
MÜHLETHALER, A. K. (1950). Electron microscopy of developing plant cell walls. Biochim. Biophys. Acta *5*, 1—9.
NANCE, J. F. (1949). Inhibition of salt accumulation in excised wheat roots by 2,4-dichlorophenoxi acetic acid. Science *109* (2825), 174—76.
NELSON, A. (1946). Principles of agricultural Botany. Nelson and Son Ltd. London.
NICKERSON, W. J. (1949). Dependence, in yeast, of phosphate uptake and polymerization upon the occurrence of glucose polymerization. Experientia V, 202—3.
NIE, R. VAN, R. J. HELDER and W. H. ARISZ (1950). Ion secretion into the xylem and osmotic regulation of exudation. Proc. Kon. Akad. Wet. *53*, 567—75.
OLSEN, C. (1938). Iron absorption and chlorosis in green plants. C. R. trav. Laborat. Carlsberg sér. chim. *21*, 15—52.
OLSEN, C. (1938). Experiments with different quantities of iron salts given to maize in water culture. Ibid. *21*, 301—14.
ÖSTERLIND, S. (1951). Anion absorption by an alga with cyanide resistent respiration. Physiol. Plant. *4*, 528—34.
OVERBEEK, J. VAN (1942). Water uptake by excised root systems of the tomato due to non-osmotic forces. Amer. J. Bot. *29*, 677—83.
OVERSTREET, R. and T. C. BROYER (1940). The nature of absorption of radioactive isotopes by living tissues as illustrated by experiments with barley plants. Proc. Nat. Acad. Sci. *26*, 16—24.
PAAUW, F. VAN DER (1948). Absorption of phosphate and nitrogen, assimilation and structural distribution in potatoes grown under varied phosphate conditions. Versl. Landb. Onderz. *54*, 3.
PARKER, F. W. and J. F. FUDGE, J. F. (1927). Soil-phosphorus studies. I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and the soil solution. Soil Science *24*, 109.
PERNER, E. S. (1952). Zellphysiologische und zytologische Untersuchungen über den Nachweis und die Lokalisation der Cytochrom-Oxydase in Allium Epidermiszellen. Biol. Zentralbl. *71*, 43—69.
PHILLIS, E. and T. G. MASON (1940). The effect of ringing on the upward movement of solutes from the root. Ann. Bot. N.S. *4*, 635—44.
PHILLIS, E. and T. G. MASON (1940). The effect of ringing and of transpiration on mineral uptake. Ibid. *4*, 645—50.

PREVOT, P. and F. C. STEWARD (1936). Salient features of the root systems relative to the problem of salt absorption. Plant Physiol. *11*, 509—34.
RICHARDS, F. J. and W. G. TEMPLEMAN (1936). Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. *50*, 367—402.
ROBERTSON, R. N. (1940). Studies on the metabolism of plant cells. I. Accumulation of chlorides by plant cells and its relation to respiration. Austr. J. exp. Biol. Med. Sci. *19*, 264—78.
ROBERTSON, R. N. (1950) Presidential address. Proc. Linn. Soc. N. South Wales *75*.
ROBERTSON, R. N. (1951) Mechanism of absorption and transport of inorganic nutrients. Ann. Rev. Plant Physiol. *2*, 1—24.
ROBERTSON, R. N. and J. S. TURNER (1945). Studies in the metabolism of plant cells. III. The effect of cyanide on the accumulation of potassium chloride and on respiration; the nature of the salt respiration. Austr. J. exp. Biol. Med. Sci. *23*, 63—73.
ROBERTSON, R. N., M. J. WILKINS and D. C. WEEKS (1951). Studies in the metabolism of plant cells. IV. The effect of 2,4 dinitrophenol on salt accumulation and salt respiration. Austr. J. Sci. Res. *B 4*, 248—64.
ROELOFSEN, P. A. and A. L. HOUWINK (1951). Cell wall structure of staminal hairs of *Tradescantia virginica* and its relation with growth. Protoplasma *40*, 1—22.
ROTHSTEIN, A. and R. MEIER (1948. The relationship of the cell surface to metabolism. I. Phosphatases in the cell surface of living yeast cells. J. cell. Comp. Physiol. *32*, 77—95.
ROUSCHAL, E. und S. STRUGGER (1940. Der fluoreszenz optisch-histo-chemische Nachweis der kutikulären Sekretion und des Salzweges im Mesophyll. Ber. d. bot. Ges. *58*, 50—69.
RUFZ DE LAVISON, J. DE (1910). Du mode de pénétration de quelques sels dans la plante vivante. Role de l'endoderme. Rev. Gen. Bot. *22*.
SABININ, D. A. (1925). On the root system as an osmotic apparatus. Bull. Inst. Recherche biol. Univ. Perm *4*, suppl. 2, 129—36.
SANDE BAKHUYZEN, H. L. VAN DE (1937). Studies on wheat grown under constant conditions. Stanford Univ. Press California.
SANDSTRÖM, B. (1950). The ion absorption in roots lacking epidermis. Physiol. Plant. *3*, 496—585.
SAYRE, J. D. (1948). Mineral accumulation in corn. Plant Physiol. *23*, 267—81.
SCHMIDT, O. (1936—37). Die Mineralstoffaufnahme der höhere Pflanze als Funktion einer Wechselbeziehung zwischen inneren und äusseren Faktoren. Zeitschr. Bot. *30*, 289—334.
SCHUFFELEN, A. C. (1948). Growth substance and ion absorption. Plant and Soil *1*, 121—26.
SCHWAB, G. M. (1941). Handbuch der Katalyse. III. Biokatalyse. Springer—Verlag Wien.
SHAPIRO, B. (1947). The mechanism of phloridzin glucosuria. The biochem. J. *41*, 151—54.
SNELL, F. D. and C. T. SNELL (1936). Colorimetric methods of analysis. Chapman and Hall Ltd. London.
STEINBACH, H. B. (1951). Permeability. Ann. Rev. Plant Physiol. *2*, 323—42.
STEWARD, F. C. (1943). The effect of ringing and transpiration on mineral uptake. Ann. Bot. N.S. *7*, 89—92.
STEWARD, F. C. (1935). Mineral nutrition of plants. Ann. Rev. Biochem. *4*, 519—44.
STREET, H. E. and J. S. LOWE (1950). The carbohydrate nutrition of tomato roots. II. The mechanism of sucrose absorption by excised roots. Ann. Bot. N.S. *14*, 307—29.
THOMAS, W. (1933). Absorption, utilization and recovery of nitrogen, phosphorus and potassium by apple trees grown in cylinders and subjected to differential treatment with nutrient salts. J. agric. Res. *47*, 565—81.
TSAO, TSUNG-HSUN G. S. RABIDEAU and W. G. WHALEY (1950). The phosphorus uptake of Andropogon ischaemum L. at various stages of development. Plant Physiol. *25*, 653—65.
WALKLEY J. (1940). Protein synthesis in mature and senescent leaves of barley. New Phytolog. *39*, 362—69.

WATSON R. and A. H. K. PETRIE (1940). Physiological ontogeny in the tobacco plant. IV. The drift in nitrogen content of the parts in relation to phosphorus supply and topping with an analysis of the determination of ontogenetic changes. Austr. J. exp. Biol. Med. Sci. *18*, 313.

WEEKS D. C. and R. N. ROBERTSON (1950). Studies in the metabolism of plant cells. VIII. Dependence of salt accumulation and salt respiration upon the cytochrome system. Austr. J. Sci. Res. *B 3*, 487—500.

WENT, F. W. and M. CARTER (1948). Growth responce of tomatoplants to applied sucrose. Amer J. Bot. *35*, 95—106.

WENT, F. W. (1944). Plant growth under controlled conditions. III. Correlations between various physiological processes and growth in the tomato plant. Amer. J. Bot. *31*, 597—618.

WIERSUM, L. K. (1947). Transfer of solutes across the young root. Rec. trav. bot. Néerl. *41*, 1—79.

WILFARTH, H., H. RÖMER und G. WIMMER (1906). Über die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchs Stat. *63*, 1—70.

WILLIAMS, R. F. (1938). Physiological ontogeny in plants and its relation to nutrition. IV. The effect of phosphorus supply on the total protein- and soluble nitrogen contents and water content of the leaves and other plant parts. Austr. J. exp. Biol. Med. Sci. *16*, 65—83.

WILLIAMS, R. F. (1948). The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineus plants. Austr. J. sci. Res. ser. *B* 1, 333—61.

WILSON, C. C. and P. J. KRAMER (1949). Relation between root respiration and absorption. Plant Physiol. *24*, 55—59.

WOOD, R. K. S., A. H. GOLD and T. E. RAWKINS (1952). Electron microscopy of primary cell walls treated with pectic enzymes. Amer. J. Bot. *39*, 132—33.

WOODFORD, E. K. and F. GREGORY (1948). Preliminary results obtained with an apparatus for the study of salt uptake and root respiration of whole plants. Ann. Bot. N.S. *12*, 335—70.