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THE ABSORPTION OF LABELLED CHLORIDE AND BROMIDE IONS BY YOUNG INTACT BARLEY PLANTS

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ABSTRACT

The techniques for cultivating the young intact barley seedlings used in these absorption experiments are described in some detail.

Sets of twelve selected seedlings were used and all data obtained refer to these units.

In the experiments, proper absorption took place from dilute solutions of either potassium chloride alone or a mixture of potassium bromide and chloride, the total concentration of which was 0.001 M. Calcium sulphate was also present in all experimental solutions.

Chloride is absorbed from the medium at an almost constant speed for a number of hours. This absorption is not influenced by subsequent distribution. At first, the root tissue tends to retain a large proportion of the chloride absorbed at the expense of the transfer of ions to the shoots. Gradually the transfer increases until a constant value is reached within a few hours.

Identical results were obtained for total halides if absorption took place from a mixture of chloride and bromide. In addition it was found that chloride and bromide were absorbed at a ratio, which was constant and about twice as high as the ratio or the ions present in the medium. This ratio remained constant even in the dark when total absorption tended to decline slightly.

In the transport processes following primary absorption, further discrimination between chloride and bromide ions takes place. The additional screening effect of the root tissue is very small. Nevertheless, important conclusions could be drawn from the behaviour of the Cl/Br ratios of the halides found in roots and shoots. For root tissue this ratio, which must equal the ratio for total absorption at the beginning of the experiment, decreases slightly until a somewhat lower but constant level is reached after a few hours. Although there is no doubt about this decrease for the root tissue, it proved to be so small that the simultaneous increase of the ratio for the shoot tissue was hardly discernable under normal conditions. It became so, if the transfer of ions to the shoot was reduced by a dark treatment.

These results could be explained by assuming that the root tissue consists of at least two compartments, differing with respect to the Cl/Br ratio. Ions are brought from the medium into the first compartment by the primary absorption process and then transferred to either the second compartment or via the xylem vessels to the shoots.

It is tempting to identify the first compartment with the symplasm of the root tissue, the second one with the vacuole system.

The process that takes ions out of the symplasm into the vacuoles prefers bromide ions to chloride ions. Little preference, if any, for either chloride or bromide ions was found for the secretion of ions from the symplasm into the xylem vessels. This is consistent with the idea that the secretion is a simple leakage of a solution out of the tissue.

Under conditions of suppressed transfer to the shoots, the accumulation of ions from the symplasm into the vacuoles becomes relatively more important. This causes greater shifts in the Cl/Br ratios found in the different compartments.

Introduction

On entering roots of intact plants, ions are subject to various absorption and translocation mechanisms. The ions already present within the root tissue are distinguishable into two fractions. This has been shown for phosphate ions in experiments on labelled phosphorus absorption by young barley plants (Helder, 1957). Those ions which could either be returned to the medium, or transferred to the shoot, or more definitely fixed within the root tissue were defined as the "exchangeable fraction". The extent of this fraction was determined, and the various fluxes between the external medium and this exchangeable fraction, and between this fraction and the fixed fraction in the root or the shoot were also determined.

In a similar way the results obtained for the absorption and distribution of labelled rubidium were analysed (Helder, 1958). In contrast to what was found for phosphorus hardly any exchange of ions could be found. Those ions in the root tissue which had just been taken up and which could be either transferred or fixed were now called the "mobile fraction".

For a further analysis of the complexity of the absorption phenomena it was thought important to trace the behaviour of the accompanying anion as well. The halide ion suggested itself as being the most appropriate one because it is rapidly absorbed and does not show the disadvantage of being as intimately involved in metabolism as are phosphate, nitrate and sulphate. Moreover, as chloride and bromide ions show mutual competitive inhibition in the absorption mechanism, (Epstein, 1953; Böszörményi and Cseh, 1958) it was tempting to study this interaction also for processes such as accumulation in the vacuoles and secretion into the xylem vessels, which are thought to be essential aspects of salt absorption and translocation in intact plants. Such a study could aid in elucidating the complex nature of the processes mentioned.

Some of the results of the more simple experiments will be given here. In the discussion an attempt is made to analyse the absorption process by using the data found in these experiments only, in order to arrive at a picture independent of that based on previous results and explanations.

Material and methods

Plant material

About 20 grams of barley seeds (cultivar. Union zomergerst) were soaked in running tap water for 20–30 minutes. The seeds were then treated with a few drops of peroxide to sterilise them, rinsed in demineralized water and allowed to germinate between two sheets of moist filter paper at 20°C for 48 hours. The seeds were germinated in a plastic box, the bottom of which was shaped in such a way that two small troughs were formed on either end of the box. Demineralized

water was poured into these troughs and the ends of the sheets of filter paper were dipped into the liquid.

Most investigators, who grow barley seedlings or excised barley roots for their experiments, use to soak the seeds for at least 24 hours. We found that such a long treatment was not only unnecessary but even depressed the number of seeds that germinated.

A little over two hundred germinating seeds were arranged on a frame in a tank as can be seen in figure 1. The frame was made mainly from ordinary plastic electric tubing. The tank was an earthenware sink, measuring 50 × 48 × 18 cm. It contained 10 litres of a Hoagland solution, which was continuously aerated. At the beginning this solution just touched the seeds, but later the level of the solution gradually became lower due to the transpiration of the seedlings and surface evaporation. This was an advantage, for it suppressed the

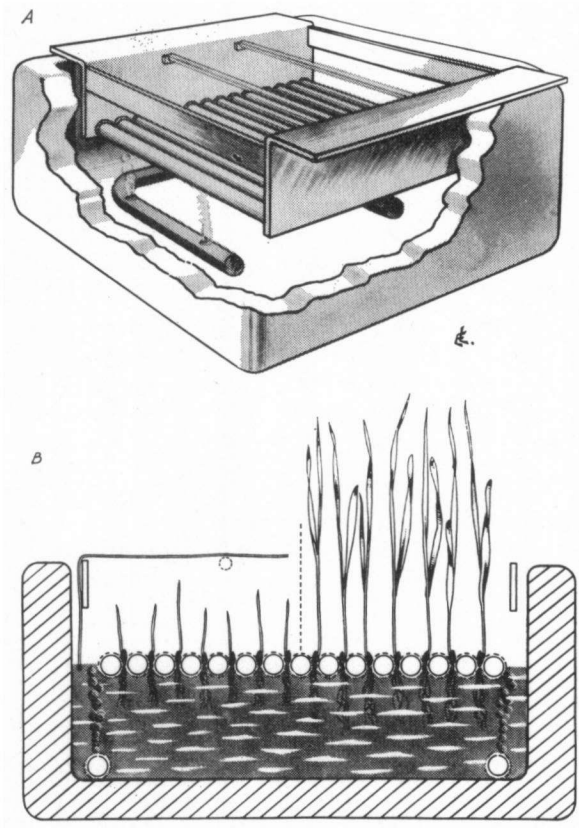

Fig. 1. The apparatus used for growing young intact barley seedlings.
A: a cut away view of tank and frame.
B: a cross section showing, on the left, the young seedlings while covered by filter paper, and on the right, the plants at the stage in which they are used in the absorption experiments.

development of fungi on the seeds and basal parts of the seedlings.

During the first 30 hours, the frame was covered by a sheet of filter paper to keep the young seedlings in a moist atmosphere (fig.1B, left-hand side). The total growing period was 7 days. The temperature was kept constant at 20°C and the seedlings were illuminated for 12 hours from 6 a.m. till 6 p.m. by four 60 Watt fluorescent tubes set at a distance of 55 cm. The light intensity was 4000–4500 lux as measured by a "Lange Standard Beleuchtungsmesser II" placed near the top of the tank. The length of the tubes was sufficient for the illumination of three tanks.

One day before the absorption experiment proper the seedlings were removed from the frame and arranged on moist filter paper according to size. This material was selected till 144 healthy looking plants were left. This group of plants was split up into 12 sets, which were made as uniform as possible by systematic randomisation.

The 12 plants of each set were then mounted into the absorption vessels which contained 500 ml of a dilute calcium sulphate solution (0.25 mM/l). The plants remained in this solution for 18 hours until the next morning, when the absorption experiment was started. They were illuminated continuously by 4 fluorescent lamps. Light conditions were similar to those described earlier, except that illumination was now continued up to the end of the absorption experiment.

A single absorption vessel is shown in figure 2. It is made entirely of perspex. It has a funnel for administering the solutions, and an outlet for emptying. In order that the vessel should empty properly, it was placed on a shelf in such a way that it could be tilted slightly.

The lid had 4 holes for the corks which carried the plants. Each cork had 3 slots into which the plants were mounted by means of a tuft of cotton wool. After the plants had been placed in the absorption

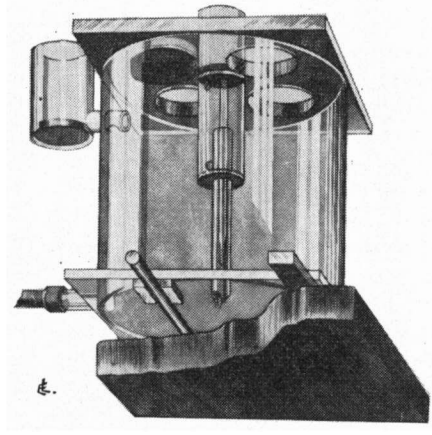

Fig. 2. The absorption vessel used in these experiments, with the special aerator for avoiding contamination of the basal parts of the plants by the radioactive solution.

vessel their position was adjusted so that the seeds were just above the surface of the solution.

Initially, the solution was aerated with the aid of a simple capillary tube, but this splashed the basal parts of the plants and the lower side of the lid which thus became contaminated with radioactive solution. To avoid this, a special aerator was constructed (figure 2). The air bubbles from the capillary tube take the solution along with them. In the wider part of the aerator air bubbles and solution are separated. The air escapes through a hole above the surface of the liquid; the solution flows back through 3 holes into the bulk of the solution. By this means the solution was aerated and circulated, and splashing was restricted to the wide part of the aerator under the rubber stopper which held the capillary tube.

The absorption experiment

Two types of experimental solution were used: either a 0.001M solution of labelled potassium chloride or a solution containing equal parts of potassium chloride and bromide and having the same total concentration. In addition to the halides the solutions always contained 0.25 mM of calcium sulphate.

The labelled chloride solution was prepared from a radioactive HCl-solution of high specific activity (0.35 mC/mg) provided by Philips Duphar N.V., Amsterdam. The acid was neutralized with potassium hydroxide and diluted. This solution contained about 12 μC Cl-36 per litre. No unlabelled KCl was added.

For the other solution, the solution just described was simply mixed with a solution of potassium bromide having the same concentration. In some experiments the bromide was labelled by adding radioactive ammonium bromide up to a concentration of about 15 μC Br-82 per litre. As the specific activity was very high the addition of this substance did not materially affect the composition of the solution.

Changing the solution in the absorption vessel was done according to a rigid time schedule and with utmost care to avoid damaging the roots as far as was possible. The old solution was removed slowly by opening the outlet and the roots were allowed to drain for one minute. A little over 500 ml water containing calcium sulphate was poured into the vessel. This took half a minute. The roots remained in this solution for one minute in order to remove the adhering old solution. The liquid was removed and the roots allowed to drain as indicated for the old solution. Then 500 ml of a fresh solution was added.

If the plants had to be harvested, the procedure was the same as indicated for changing the solution, but instead of adding a fresh solution the plants were removed from the corks, the roots cut and put into 150 ml beakers. The shoots were cut into pieces and also collected in similar beakers.

Analytical procedures

Various methods for the determination of chloride and bromide in

experimental solutions as well as in plant extracts have been used. Mohr's method was used for the determination of chloride or total halide content of the experimental solutions. This could be done with reasonable accuracy if the solutions were concentrated 4–5 times.

The chloride content of the both solutions and plant extracts could also be determined by counting radioactivity, provided that the specific activity was known. This was the case for the experiments discussed here, where all solutions were prepared from the same stock solution without the addition of unlabelled chloride. For the determination 10 ml samples were poured into a cylindrical container into which a simple dipping counter (20th Century Electronics G.M. type 2H) was mounted. A ten-times diluted experimental solution was used as a reference.

If Br-82 was also present in the solutions it interfered though only slightly. For that reason the determination of the Cl-36 activity was postponed then for at least 14 days. The activity of the bromide had almost disappeared by then as its half-life is only 36 hours.

In these experiments bromide was always accompanied by chloride, and its concentration could be calculated as the difference between total halide and chloride content. For a direct determination a method based on König's reaction was used (Van Pinxteren, 1952).

Bromide was converted to BrCN. This in turn was led into a mixture of pyridine and benzidine, and a red compound was formed. This was dissolved in alcohol and made up to volume in a 50 ml volumetric flask.

This very sensitive method gave good reproducible result, even in the presence of large amounts of chloride. One drawback was the instability of the colour at room temperature. All reagents were therefore kept at −10°C and the intensity of the coloured solution was determined as quickly as possible.

Another disadvantage of this method is the strong interference by even minute quantities of organic matter, and consequently, plant material had to be ashed, as will be described in more detail below. Moreover, special precautions also had to be taken with regard to the experimental solutions. Because of the long half-life of the radioactive chloride, and also for economic reasons, the solution was used for several absorption experiments. As a result excretion products from the roots tended to accumulate slowly in the course of time. After the solution had been concentrated, these appeared as a yellowish substance which proved to interfere with the chemical bromide determination. Fortunately, this difficulty could be overcome by pouring the solution back into the container through a carbon filter each time it had been used in an absorption experiment.

In cases where labelled bromide solutions were used, the content was determined by counting the radioactivity of 5 ml samples using a scintillation counter with a well-type NaJ-crystal. The radioactive chloride, which was always present, did not interfere.

In order to determine the halide content of the plants the root and cut shoot material was extracted four times in 30 ml of a boiling alka-

line 0.1 % NaCl solution. This procedure removed all the labelled chloride and bromide, and could easily be checked by testing the radioactivity of the residue. The extract was filtered into 50 ml flasks and made up to volume after cooling.

If dry ashing was necessary, the plant material was collected in silica basins. Halides can not be determined without the addition of a basic substance. Therefore, a suspension was prepared containing 5 gram of calcium oxide per litre. About 5 ml of this suspension was added to the root material whereas 20 ml was added to the shoot material. The content of the basins was subsequently dried and then ashed for one hour at a temperature that was raised gradually from about 200°C up to 500°C. Finally, the ash was dissolved with the aid of a few ml sulphuric acid 1N, and diluted to known volume.

Statistical aspects

It has been indicated how the seedlings were grown in order to obtain material as homogenous as possible. By sticking to a rigid time schedule good results were obtained. For instance, in earlier experiments the appearance of the root systems might vary considerably from one experiment to the other. In one batch long thin roots might be formed, whereas in the next experiment roots might be short and thick with numerous root hairs.

In the experiments reported here all root systems were well developed with long and short roots all covered with root hairs to a certain extent.

The shoots were in the second leaf stage and their development was very much the same in successive experiments. However, in spite of the constant conditions of the constant-temperature room there was a clear seasonal trend, as the shoots were shorter during the winter season. Therefore, in each experiment the average lengths of the first and the second leaf of the twelve plants of a single set were measured and recorded. For the material used in the experiments reported here, we found these lengths on average to be 19.9 cm for the first leaf and 15.2 cm for the second leaf. As we have not found any qualitative relationship between shoot development and experimental results, we have not given the shoot development in the experimental data.

The variability of the experimental data is best illustrated by the results of two simple absorption experiments shown in fig. 3. It can be seen that, on the whole, fairly good results could be obtained. However, in some instances (e.g. the 9 hours absorption data in fig. 3B) irregularities arose as a result of a single exceptionally high or low value. In the earlier experiments these values were discarded, but on the basis of the results of a few hundred experiments done in our laboratory during the past decade we have dropped this procedure. If there has been doubt as to the meaning of the results because of the variability just mentioned, the experiment was repeated at least 3–5 times until a consistent picture was obtained in all experiments.

If necessary, the number of treatments within a single experiment was reduced to 3 or even 2. As 12 sets were used in all experiments, this means that the mean values given have been calculated from 4 or even 6 replicates.

EXPERIMENTS

The course of absorption of labelled chloride

The analysis of absorption and distribution processes is made easier if these processes have reached a more or less constant rate. For this reason the time course of chloride absorption was studied for periods of up to twelve hours. Typical results are shown in fig. 3A and fig. 3B. At three-hourly intervals three sets of plants were harvested and the chloride content of roots and shoots determined. The individual figures are given. As to the variability of the results we refer to the section "Statistical aspects".

Because of this variability it was found difficult to determine the exact course of total absorption, and a number of additional absorption experiments in which the number of treatments was reduced were therefore, carried out. In all these experiments some of the sets of plants were allowed to absorb for six hours. The amount absorbed by these sets was then used as a reference value and put equal to 100 in order to be able to compare results obtained in subsequent experiments. The results are summarized in Table 1.

It appears from this table that the rate of absorption from the medium is virtually constant for the first six hours. At any rate, if there is anything like a rapid initial uptake, it is completely masked by the high rate of subsequent absorption.

In most of the experiments the absorption showed a temporary

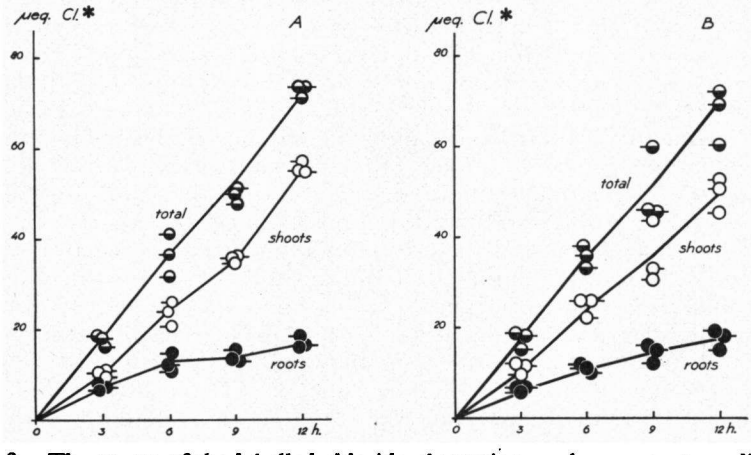

Fig. 3. The course of the labelled chloride absorption, under constant conditions of light, temperature and composition of the solution, by sets of twelve intact barley plants.

TABLE 1

The course of the absorption of labelled chloride ions during the first twelve hours.
Sets of 12 barley plants were allowed to absorb from a KCl 0.001 M solution for the period indicated. The total amount absorbed was determined and expressed as a percentage of the 6-hour value.
Each experiment was carried out with twelve sets of plants.

Experiment	Absorption-period 3 hr.	6 hr.	9 hr.	12 hr.
1	49 %	100 % 36.7 μeq Cl	139 %	193 %
2	49 %	100 % 35.8 μeq Cl	141 %	189 %
3	48 %	100 % 34.4 μeq Cl		
4	51 %	100 % 31.6 μeq Cl	153 %	
5		100 % 31.0 μeq Cl	144 %	
6		100 % 30.1 μeq Cl	145 %	
7		100 % 28.0 μeq Cl		202 %
"Average"	49 %	100 %	144 %	195 %

slowing down after these first six hours. However, during the last three-hour period, absorption increased to its initial rate. In a single experiment not given here, in which absorption took place from a 2 mM solution, it was found that absorption may continue at a constant rate for 24 hours.

Such an almost constant rate of absorption, interrupted by a short interval of reduced uptake, had also been found in earlier experiments on the absorption of labelled rubidium (HELDER, 1959). In this case, reduced uptake was ascribed to periodicity phenomena and also to an additional effect exerted by the rubidium ion itself. In the light of the present results on chloride absorption, the second assumption seems to be rather less likely unless the potassium ions have a similar influence.

A satisfactory explanation cannot yet be given. The phenomenon may be influenced by a number of factors such as the diurnal rhythm during the cultivation of the material, the time of placing the plants into the absorption vessels, the time of starting the experiments etc. It should be realized that the factors mentioned may have an indirect influence. It is conceivable that transpiration is reduced during the afternoon as a result of a periodical closing of the stomata, and this reduction of transpiration will then suppress the salt absorption.

The distribution of chloride ions between the roots and shoots can be seen in fig. 3A and fig. 3B. The data suggest that at first, much of the chloride absorbed is retained by the roots, and that transfer to the shoots becomes more significant after a couple of hours. However, it is difficult to ascertain the exact course of the distribution processes, because at the beginning of the experiment the situation is affected

by the fact that the roots have first access to the absorbed ions, and during the second part of the experiments the results are marred by the temporary depression of the absorption from the medium. Clearly, for a better understanding of the distribution processes these simple absorption experiments were inadequate.

The course of absorption from a mixture of chloride and bromide

If the potassium chloride solution used in the previous experiments is replaced by a solution containing a mixture of equal amounts of chloride and bromide, absorption and distribution of both chloride and bromide can be studied as well as any interaction of these ions. In five experiments the time course was tested by determining the salt content after three- and six-hour absorption periods. Mean values are summarized in Table 2.

It is apparent from this table that the rate of chloride absorption is constant for the first six hours under the present conditions also. The same applies to the bromide absorption, the only difference between chloride and bromide absorption being the much faster absorption of the chloride ion.

In the medium, the chloride and bromide ions are present in equal concentrations. If there was no preference for either of them, the same rate of absorption for these two different halide ions could be expected. Actually, the chloride ion is absorbed at a rate about twice as high as that of the bromide ion. The extent of the preference for

TABLE 2

The course of the absorption of labelled chloride and unlabelled bromide ions.

In five separate experiments, sets of twelve barley plants were allowed to absorb from a 0.001 M solution containing KCl and KBr at equal concentrations. At the end of the absorption period indicated, both the chloride and bromide contents of roots and shoots and thus the content of the whole plants, were determined. As the results of the individual experiments were similar, only the average values are given.

From these data both the ratio and the sum of the chloride and bromide contents were calculated. The results are indicated as "halide ratio" and "total halide".

	Absorption-period 3 hours			
	chloride	bromide	ratio	total halide
roots	4.10 μeq	2.09 μeq	1.96	6.19 μeq
shoots	4.01 μeq	1.77 μeq	2.26	5.78 μeq
total	8.11 μeq	3.86 μeq	2.10	11.97 μeq

	Absorption-period 6 hours			
	chloride	bromide	ratio	total halide
roots	5.96 μeq	3.41 μeq	1.74	9.37 μeq
shoots	10.11 μeq	4.41 μeq	2.29	14.52 μeq
total	16.07 μeq	7.82 μeq	2.05	23.89 μeq

chloride relative to bromide can, therefore, be expressed by a factor of two.

As in the previous section, little can be said about the rate of the distribution processes. But it can be seen that the chloride-bromide ratio for the halides found in the root tissue is lower than the value found for the absorption from the medium. This is reflected by the value for the shoots, where it is higher than for absorption from the medium, so, the root tissue has, therefore, a small screening effect in addition to that displayed by the outer barrier.

Finally, it can be seen that halide ratio found for the root tissue is lower after six hours absorption than after three hours. This was so in all five experiments. However, little can be said about a change in this ratio with regard to the shoots and total absorption, as the results of the five experiments were not consistent in this respect. Clearly, if this ratio is constant for total absorption, the ratio for the halides found in the shoots should show some increase. It can be calculated that this increase would be small because the amount of halides transferred to the shoots is large, as compared to the amount retained in the roots. Obviously, these experiments do not enable one to prove the significance of such a small increase.

The absorption of labelled chloride after a treatment in unlabelled chloride

The course of the ability of the plants for chloride absorption can also be studied by allowing the plants to absorb labelled chloride for a fixed period after they have been pre-treated in unlabelled solutions for varying periods of time. The results of two such experiments are given in fig. 4A and fig. 4B. In the first experiment some sets were put directly in a labelled solution for three hours, other sets after they had been in an unlabelled solution for three or six hours. In the second experiment these periods amounted to six and twelve hours

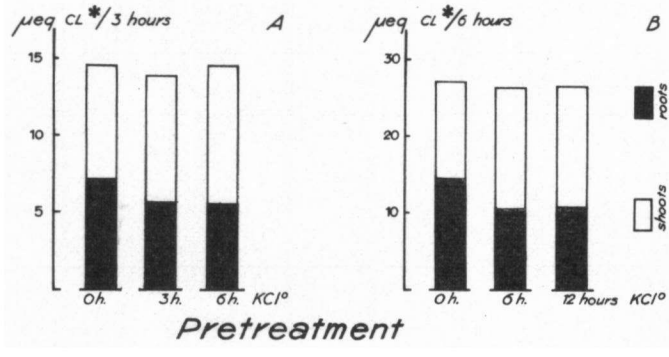

Fig. 4. The absorption and distribution of labelled chloride after a pre-treatment in an unlabelled solution for various periods.
A. 3-hour absorption period.
B. 6-hour absorption period.

whereas absorption of labelled chloride took place for six hours.

It can be seen that the total amount of labelled chloride taken up is not affected by the duration of the pre-treatment. This means that the rate of chloride absorption was constant, as it was in the experiments previously discussed. More information can be obtained from these experiments with regard to distribution. As all sets had been in a labelled solution for the same period of time, the amounts of labelled chloride found either in the root tissue or the shoot tissue can be compared directly. It is observed that the amount of labelled chloride transferred to the shoots is increased after a pre-treatment in an unlabelled solution but reaches a constant value within a few hours.

In order to trace the course of this change in the distribution pattern a number of experiments were carried out similar to that of fig. 4A, but with pre-treatment periods varying from zero to nine hours. The results are shown in Table 3. In these experiments a slight decrease was found in the amount of total absorption.

The change in the distribution can be deduced from the percentage of the ions absorbed, that is transferred, to the shoots. Obviously, the bigger change happens during the first few hours. A pre-treatment period longer than three hours causes very little, if any, further increase of the amounts of chloride ions transferred to the shoots. Thus, in these experiments also the absorption from the medium by young intact plants appears to proceed at a fairly constant rate within the time of the experiments. Strikingly, no depression of the absorption rate was found for the six to nine hours period.

This constant rate of absorption is in obvious contrast to the considerable change in the distribution of the ions absorbed. It follows that the absorption is independent of the processes governing the distribution and that the ultimate fate of an ion taken up is not yet determined at the very first stage of the absorption process.

TABLE 3

The influence of a pre-treatment in unlabelled KCl solution on subsequent absorption and distribution of labelled chloride.

Sets of twelve barley plants were allowed to absorb from a 0.001 M labelled KCl solution for three hours after they had been in an unlabelled solution of similar composition for various periods.

The figures are based on six experiments. In addition to the absolute values, the percentages of the total amount of the absorbed labelled chloride found in roots and shoots are indicated. These percentages demonstrate the change in the distribution.

	Pre-treatment period							
	0 hours		3 hours		6 hours		9 hours	
roots	8.7 μeq	57 %	6.7 μeq	46 %	5.6 μeq	41 %	5.6 μeq	42 %
shoots	6.5 μeq	43 %	8.0 μeq	54 %	8.0 μeq	59 %	7.6 μeq	58 %
total	15.2 μeq	100 %	14.7 μeq	100 %	13.6 μeq	100 %	13.2 μeq	100 %

The simultaneous absorption of labelled chloride and bromide after a pre-treatment in unlabelled halide solution

In these experiments the plants were allowed to absorb ions from a solution containing a mixture of equal amounts of labelled chloride and labelled bromide, after they had been pre-treated in unlabelled solutions of the same composition. In three experiments a three-hour pre-treatment was compared with no pre-treatment. In five more experiments such a three-hour pre-treatment was compared with a six-hour pre-treatment. As the results within each set of experiments were alike, only the average results are given in Table 4.

As was found in the experiments on labelled chloride absorption, total absorption of halide was not significantly altered by a three- or six-hour treatment in unlabelled solution prior to the absorption of the labelled ions. So the rate of absorption from the medium is found to be constant again. The distribution of the absorbed ions, however, changes markedly during the first three hours. In this respect also, the results are consistent with those for chloride absorption.

From the data on labelled chloride and bromide content of roots, shoots and whole plants, the chloride-bromide ratios can be calculated. This ratio does not show any apparent trend for total absorption. In the individual experiments it was found that after a pre-treatment in unlabelled solution, the ratio could be either slightly higher or lower than the ratio for the control. For the root tissue on the other

TABLE 4

The influence of pre-treatment in an unlabelled solution of KCl and KBr on subsequent absorption, distribution and interaction of labelled chloride and bromide

Sets of twelve barley plants were allowed to absorb from a 0.001 M solution of labelled KCl and KBr at equal concentration for three hours after they had been in an unlabelled solution of similar composition for various periods. A: the average result of 3 experiments with pre-treatment periods of 0 and 3 hours. B: the average result of 4 experiments with pre-treatment periods of 3 and 6 hours.

A	Pre-treatment period: 0 hours					3 hours				
	chloride	bromide	halide ratio	total halide		chloride	bromide	halide ratio	total halide	
	μeq	μeq		μeq	%	μeq	μeq		μeq	%
roots	3.09	2.02	1.53	5.11	45	2.15	1.53	1.41	3.68	34
shoots	4.06	2.06	1.97	6.12	55	4.77	2.36	2.02	7.13	66
total	7.15	4.08	1.75	11.23	100	6.92	3.89	1.78	10.81	100

B	Pre-treatment period: 3 hours					6 hours				
	chloride	bromide	halide ratio	total halide		chloride	bromide	halide ratio	total halide	
	μeq	μeq		μeq	%	μeq	μeq		μeq	%
roots	2.26	1.61	1.40	3.87	34	2.09	1.54	1.36	3.63	33
shoots	4.80	2.62	1.83	7.42	66	4.85	2.68	1.81	7.53	67
total	7.06	4.23	1.67	11.29	100	6.94	4.22	1.65	11.16	100

hand there was a significant decrease after a three-hour pre-treatment. This corroborates the results found in the simple absorption experiments discussed in the second section.

As to the ratio for the halides found in the shoots the data are less convincing. On average there is a very slight increase as was found in the previous absorption experiments. Moreover, this increase, however small, was found in all experiments. From this it seems justified to conclude that this ratio tends to increase during the first few hours. The result should also be considered against the background that the ratio for the root tissue shows a decline during this period.

Nevertheless, it remained desirable to modify experimental conditions in order to obtain a more clear-cut picture. For that reason a few experiments were done in which the transfer of ions to the shoots was suppressed by putting the plants in the dark during the absorption experiment proper. If the chloride-bromide ratio is constant for the absorption from the medium and this ratio decreases for the ions retained in the root tissue, a corresponding increase for the ions transferred to the shoot should become more apparent under these conditions.

As is shown by Table 5 this is what was actually found. Here absorption from the medium shows a slight decrease, obviously as a result of the dark treatment; more striking is the constant value found for the ratio of the chloride and bromide ions taken up. The amounts transferred to the shoots are lower than those in the experiments carried out in the light, but the change in the distribution after a three-hour pre-treatment is equally apparent. The chloride-bromide ratio for the halide ions transferred to the shoots increases at the same time, to an even somewhat greater extent than the simultaneous decrease of this ratio for the ions in the roots.

To sum up, one can say that if young barley seedlings are allowed to absorb a mixture of chloride and bromide ions, total absorption proceeds in the light at a constant rate for a number of hours. Chloride ions are preferred to bromide ions. There is no change in this preference even when absorption slows down owing to a dark treatment. Mainly during the first three hours the distribution of the ions absorbed

TABLE 5

The influence of a pre-treatment in unlabelled solution on subsequent absorption, distribution and interaction of labelled chloride and bromide in the dark.

Details are similar to those given for Table 4A. with the exception of the light conditions. In order to reduce translocation to the shoots the plants were kept in the dark during the entire absorption period.

	Pre-treatment period									
	0 hours					3 hours				
	chloride	bromide	halide ratio	total halide		chloride	bromide	halide ratio	total halide	
	μeq	μeq		μeq	%	μeq	μeq		μeq	%
roots	4.47	2.25	1.99	6.72	57	2.91	1.64	1.77	4.55	44
shoots	3.58	1.57	2.28	5.15	43	4.10	1.60	2.56	5.70	56
total	8.05	3.81	2.11	11.86	100	7.01	3.24	2.16	10.25	100

is changed in that an increasing proportion is translocated to the shoots.

This change is accompanied by a change in the chloride-bromide ratio of the ions found in the root and the shoot tissue. If the translocation to the shoots is slowed down, a more pronounced increase of this ratio for the ions in the shoots is obtained. The significance of these findings in our understanding of the mechanism of absorption and distribution will appear from the general discussion.

Discussion

In the experiments on the time course of halide absorption it was found that the rate of absorption remained constant, or almost so, for a number of hours, in spite of the fact that the simultaneous distribution of the ions absorbed might change considerably. This had also been found in experiments on rubidium absorption (Helder, 1958).

These results are important because it is conceivable that there exist absorption sites from which ions can be moved only to the xylem vessels. Such a mechanism could explain the instantaneous reduction in the rate of bleeding of tomato roots on withdrawal of ions from the medium (Van Andel, 1953). In fact, the idea has been put forward by Bange and Van Vliet (1961) for explaining their results on potassium-sodium interactions in translocation studies with maize plants.

The results obtained in these experiments do not invalidate this concept; but even so, they do rather point in another direction, namely that the ultimate fate of ions absorbed is not already determined at the very first step of the absorption process.

If absorption took place from a mixture of chloride and bromide, these ions were absorbed at a constant ratio, of about 2, even under conditions of reduced transpiration, i.e. in the dark when total absorption slowed down a little. This stresses once more the independence of the process that brings ions from the medium into the plant tissue. Whether this is true under other conditions e.g. at a higher salt status of the tissues still requires further investigation.

Both the constant rate of absorption and the constant value of the chloride-bromide ratio indicate that there was no return of ions absorbed to the medium. In other words, no appreciable exchange occurred under the constant conditions of these experiments.

As to the distribution of the ions throughout root and shoot tissue, it was found that during the first few hours an increasing proportion of the ions absorbed are transferred to the shoots until a constant distribution pattern is attained. This may be due to the halide ions being held in the root tissue at the expense of the stream of ions across the root tissue to the xylem vessels during the first few hours of the experiment. It may also be the result of an increasing activity of a secretory mechanism which moves ions into the vessels, this increase in turn, resulting from the increasing salt level of the root tissue, which soon reaches a non-limiting value.

More complicated and more difficult to explain are the results for

distribution, if the chloride-bromide ratios are taken into consideration. For root tissue this ratio was always lower than the ratio found for total absorption. Moreover, it decreased during the first few hours. For shoot tissue the reverse was found, though the increase was very slight under normal conditions of high transfer of ions to the shoots. This simultaneous decrease for the roots and increase for the shoots makes it virtually impossible to consider the root tissue as being a one-compartment system with respect to the chloride-bromide ratio. Rather, we have to assume that the ions transferred to the shoots are derived from a pool of ions, which also shows that slight increase of the chloride-bromide ratio, in contrast to the simultaneous decrease of this ratio found for the total halide ions present in the root tissue. Clearly, the root tissue represents a system of at least two compartments. In one compartment the chloride-bromide ratio has to be lower, and in the other one higher, than the average value.

Splitting up the root tissue into two compartments can be done in numerous ways, but all the possibilities are based on two main schemes. In one scheme the ions which are translocated to the shoots have to pass through both compartments, in the other these ions pass through a single compartment only. In order to avoid too much abstract reasoning two obvious examples, each based on one of these basic schemes, will be discussed.

Let us first assume that the tissues of the cortex and the stele differ with respect to the chloride-bromide ratio as a result of an endodermal transfer mechanism, which discriminates between chloride and bromide ions, in addition to the absorption mechanism that pumps ions from the medium into the cortical cells and the secretion mechanism that regulates the movement of ions out of the tissue into the xylem vessels. It was found that the absorption mechanism prefers chloride to bromide ions. Consequently the chloride-bromide ratio was about two for the ions absorbed instead of one, the ratio for the ions in the medium. So, for the first amounts of halide ions found in the cortical tissue the ratio will also be about two. If now the transfer mechanism also prefers chloride to bromide ions, the ratio for the ions present in the tissue of the stele will show a value higher than two. However, owing to the facilitated transfer of chloride ions, the ratio for the halide ions present in the cortex will tend to decrease until a steady state is reached.

Thus, it is found that the chloride-bromide ratio for the ions present in the stele will be higher than two (the value for absorption from the medium), but it will also show a decline to a constant level.

Now if the secretion mechanism did not show any preference for either chloride or bromide ions, the flux of ions to the shoots would reflect the conditions just mentioned for the stelar tissue. However, the experimental evidence pointed to a slight but significant increase in the chloride-bromide ratio. This can only be explained by assuming a preference for bromide ions for this mechanism, as this will tend to increase the ratio for the ions left behind in the stelar tissue.

In a similar way we have to accept a preference for chloride by the

secretion mechanism if we assume the transfer mechanism to prefer bromide to chloride ions. Which of the possibilities is the most likely can be deduced from what was found about the influence of reduced transfer on the chloride-bromide ratio. If the secretion mechanism prefers chloride to bromide it will tend to reduce the chloride-bromide ratio for the tissue of the stele. The extent of this reduction will depend on the amounts of ions transferred to the shoot. If this transfer is suppressed by a dark treatment, the ratio for the stelar tissue will remain at a higher level and consequently this will also be the case for the ions removed from this tissue and transferred to the shoots. As this is what was actually found, we have to conclude that this second possibility would be the right one.

The conclusion arrived at is that it is possible to explain the observed facts in this way, at least from a semi-quantitative point of view. However, the assumption that there is a transfer mechanism which prefers bromide ions in contrast to the absorption and secretion mechanisms which prefer chloride ions, renders this scheme rather arbitrary. This impression is stressed by realizing that a number of additional assumptions have to be made with regard to the quantitative aspects e.g. the extent of the preference, the amounts of ions transferred and secreted in relation to the amounts retained in the cortical and stelar tissues etc. The exact relationships between all these factors can only be given after a more rigid mathematical analysis, but it does not seem appropriate to give all the details here, in view of the much simpler explanation given by the following scheme.

As a second example of a two compartment system we may think of the symplasm of the root tissue on one side and the vacuolar system at the other side.

Ions taken up into the symplasm will now be either transferred directly into the xylem vessel or accumulated in the vacuoles. The salt status of the symplasm is then regulated by the combined influences of the secretion and accumulation mechanisms. One of the results should be a slight increase in the chloride-bromide ratio for the ions in the symplasm. This means that bromide is preferred to chloride in the processes that remove halide ions from the symplasm. This must mainly be due to the accumulation process, in view of the effect that a dark treatment has on the change in the chloride-bromide ratio. If the secretion process showed a marked preference for bromide ions, a slowing down of this process would cause a higher bromide content of the symplasm. And this, in turn, involves a lower value of the chloride-bromide ratio for the ions translocated to the shoots.

It is even doubtful, if this process that moves ions from the symplasm into the xylem vessels shows any preference for bromide ions at all. If this were so, we would expect that at the start of the experiment, this ratio would be lower for the ions translocated to the shoots than for the ions absorbed from the medium. In fact, it was found that the former ratio is always slightly higher than the latter. Therefore the figures rather suggest that the secretion process does not display any preference at all for either bromide or chloride.

If this conclusion is right, it is even possible to make a few simple calculations. For it means that the chloride-bromide ratio for the ions in the symplasm is equal to that for the flux of ions to the shoots. So if it was found that after 3 hours of absorption the chloride-bromide ratio had reached a steady level of 2.02 for the shoots (Table 4A) it could be concluded that the ions in the symplasm of the roots must have shown the same ratio. This value has to be compared with the ratio 1.41 found for all the ions present in the root tissue. The preference of the accumulation mechanism for bromide ions is yet more clearly shown by considering the reciprocal values i.e. the bromide-chloride ratios. They are 0.50 for the symplasm and 0.71 for the whole root tissue.

If it is supposed for a moment that the amount of ions present in the symplasm is negligible as compared with those present in the vacuoles, we arrive at the conclusion that bromide ions are preferred 1.4 times in the process of accumulation. This factor increases to 2.0 if it is supposed that equal amounts of halide ions were present in the symplasmatic and vacuolar compartment.

In the same way the preference can be calculated in the experiments done under dark conditions i.e. at reduced transfer to the shoots (Table 5). Here we found 1.4 and 2.0 respectively. These results seem convincing enough to show that all the phenomena found in these experiments can be explained without any difficulty by this two-compartment model. This model lends itself to a more refined analysis. From this it appeared that the quantitative relationships depend on such factors as the proportion of ions absorbed which are retained in the symplasm, the ratio between the amounts of ions accumulated and transferred, the preference for either the chloride or bromide ion shown by the secretion and accumulation mechanism relative to that for the absorption from the medium. However, it will be clear from the crude calculation given above that we are lacking information about the distribution of the ions over the two root compartments. Hence there is no point in entering into closer detail of such an analysis.

There is one finding, even at this stage of the analysis, that needs to be stressed. As to the secretion of ions from the root tissue into the xylem vessels hardly any preference could be demonstrated. Taking into account the preference for bromide shown by the accumulation mechanism, the somewhat higher value of the chloride-bromide ratio for the ions translocated to the shoots is easily explained. This means that the mechanism that secretes ions out of the root tissue into the vessels differs essentially from the mechanism that brings ions from the medium into the tissue. As a matter of fact it can be considered to prove that secretion merely involves a leakage of ions from the tissue into the vessels, i.e. the sap in the vessels is identical with the liquid phase of the symplasm with which it forms one continuous whole.

This conclusion also demonstrates how difficult it is at this stage of our analysis to identify the two compartments with definite structures in the root tissue. It should, therefore, be realized that this dis-

cussion started merely from an example, though a very obvious one, of a two-compartment system.

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