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PRETREATMENT AND CHLORIDE UPTAKE IN VALLISNERIA LEAVES

INFLUENCE OF LIGHT, SUGAR, SALTS AND pH ON SUBSEQUENT CHLORIDE ACCUMULATION

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CHAPTER 1

INTRODUCTION

The uptake of salts by plantcells is controlled by many internal and external factors. Some investigators distinguish two phases in the salt uptake, i.e. 1° an initial non-metabolic process, which may be adsorption, adsorption-exchange or exchange process, (BROYER, OVERSTREET 1940; JACOBSON, OVERSTREET 1947; SUTCLIFFE 1952; EPSTEIN 1954, 1956); 2° one or more metabolic processes which are dependent on aerobic respiration and in which accumulation against the concentration gradient can take place (STEWARD, HARRISON 1939; V. D. HONERT 1933, 1955; ARISZ 1945, 1953, 1958; LUNDEGÅRDH 1949, ROBERTSON 1951).

Addition of sugars gives an increase in uptake. The phosphate uptake by maize plants was increased by addition of glucose (HELDER 1952). Sugars likewise increase the chloride uptake in *Vallisneria* leaves (ARISZ and SOL 1956).

Various other factors during the uptake, such as light (HOAGLAND *a.o.* 1927; JACQUES, OSTERHOUT 1934; INGOLD 1936; ARISZ 1943, 1947;

ARISZ and SOL 1956; VAN LOOKEREN CAMPAGNE 1957) and the presence of certain ions (VIETS 1944; EPSTEIN 1953; EPSTEIN and HAGEN 1952; TANADA 1955, 1956; LEGGETT and EPSTEIN 1956; OVERSTREET and others 1952; OVERSTREET 1957) appear to have an influence on the uptake of salts.

Varying these factors during the cultivation of the material and shorter pretreatment periods has also an influence on subsequent ion absorption. HOAGLAND and BROYER (1936, 1942) found that excised barley roots only absorb K and Br when they are in a low salt condition, i.e. when they contain a high percentage of carbohydrates and a low percentage of salts. The roots in a high salt condition absorb much less. ALBERDA (1948) investigated the relationships between concentration and phosphate absorption in maize plants. He found that maximum absorption was reached at concentrations which were different for high and low salt plants. This was determined by factors such as the preceding salt supply and carbohydrate metabolism.

In the uptake experiments with *Vallisneria* leaves the pretreatment of the material proves to be significant. A pretreatment with light increases the chloride uptake (ARISZ 1947; ARISZ and SOL 1956). The same holds good for a pretreatment with sugar (ARISZ and SOL).

The aim of this research was to gather an impression of the influence of different factors during the pretreatment on the rate of chloride uptake by *Vallisneria* leaves. Succesively the influence of the following factors was examined; light, sugar, carbon dioxide, salts and the hydrogen ion.

CHAPTER 2

MATERIAL AND METHODS

Vallisneria spiralis was cultivated in concrete tanks (110 × 110 × 50 cm) in the basement of the laboratory. The plants were put with their roots in clay. The tanks were filled with demineralized water. Replenishment of the vaporized water took place with it too. Illumination took place with the aid of PHILIPS HO 450 mercury vapour lamps for a daily period of 15 hours. The temperature of the room and consequently of the water amounted to 20–24° C.

Four leaves of 40 cms length were used for the experiments. From the margins of these leaves so much was cut off that everywhere a width of 4 mms was left. This was done with a specially constructed apparatus consisting of two parallel razor blades placed at 4 mms' distance from each other. Next each leaf was divided with a sharp knife into 8 parts of 5 cms length. These parts were distributed among four series in such a way that each series contained two parts from each leaf and also a segment near the top, a more basal part etc. In this way four series behaving equally, were obtained. These series of 8 leaf lengths were put in a perspex holder (Fig. 1 B) and transferred to the perspex pretreatment vessels of about 150 ml capacity. In experiments, in which all series get an equivalent pretreatment for

the first 24 hours, they were put in one large pretreatment container made of perspex. During the pretreatment the medium solution was constantly aerated with CO_2 free air. For these experiments deionized water has always been used as pure water. During the pretreatment illumination took place with a 100 Watt incandescent lamp at 40 cms distance from the vessels (100 foot-candle).

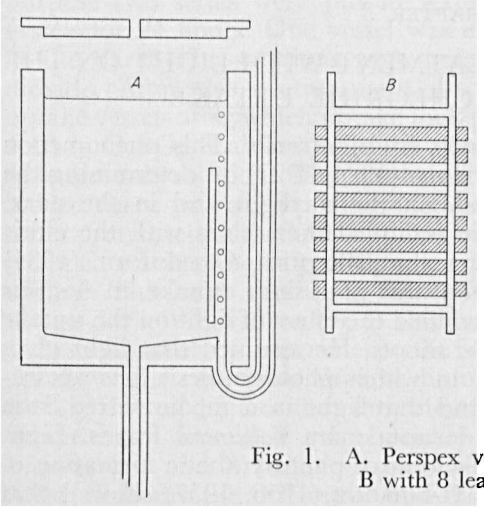


Fig. 1. A. Perspex vessel with aeration in which a frame B with 8 leaf segments can be placed.

After the pretreatment the holders with leaf segments were just washed with water and next rapidly transferred to the uptake vessels. These uptake vessels, as pictured in Fig. 1 A could contain 20 ml. The lid was taken from the vessel and the vessel was filled with the aid of a pipette. The solution was aerated with air free from CO_2 to prevent the formation of carbohydrates by photosynthesis. At the same time the airstream attended to the solution being stirred well. The vessels stood on a 20 cms high perspex stand constructed for this purpose, which made it easy to collect the solution in a beaker placed under the vessel. Illumination occurred, just as in the case of the pretreatment with an 100 Watt incandescent lamp at 40 cms distance (100 foot-candle). The whole apparatus was mounted in a constant temperature room (24° C). After the one hours uptake period the solution was collected in a beaker and twice a quick washing with water took place. This water was collected in the same beaker. The chloride present in this solution was determined and compared with the chloride content of the salt solution before the uptake period. The difference in chloride content of these salt solutions showed how much chloride had been absorbed by the leafsegments.

The chloride content of the salt solution was determined by the electrometric method, as given by BEST (c.f. PIPER, Soil and Plant analysis). For the titration a 0,01 M $AgNO_3$ solution was used. By this method the chloride present in the solution can be determined accurately to 3,5 μg Cl.

The temperature of the solution in the uptake vessels rose 0,5–1° C during the one-hour period of uptake in consequence of the heat radiation of the lamp. The rise was identical for all four vessels. This was checked by placing small thermometers in the vessels in a couple of experiments.

CHAPTER 3

INFLUENCE OF A PRETREATMENT WITH LIGHT ON THE SUBSEQUENT CHLORIDE UPTAKE

Light affects the uptake of salts by plant cells. This phenomenon was first observed by HOAGLAND *a.o.* (1924, 1927) on determining the chloride uptake of *Nitella clavata* in the daylight and in the dark. Several other investigators have occupied themselves with the effect of light on the salt uptake after that. JACQUES, OSTERHOUT (1934) examined the effect of light on the potassium uptake in *Valonia macrophysa*. INGOLD (1936) determined the effect of light on the uptake of KCl and KH_2PO_4 by *Helodea* shoots. He assumed that light gives an active uptake of substance, in which photosynthesis acts an important part. ARISZ (1943) found that light in a medium free from CO_2 also stimulates the chloride uptake by *Vallisneria* leaves. Light therefore need not act via the system of photosynthetic formation of carbohydrates. VAN LOOKEREN CAMPAGNE (1956, 1957) showed that the action-spectrum of salt uptake corresponds with that of photosynthesis. This indicates that in either process light absorption is effected by the chlorophyll. The light energy would be converted in the chloroplasts into some form of chemical energy which is direct or indirect used in the active chloride uptake.

Not only during the uptake light affects the chloride uptake in *Vallisneria* leaves. A pretreatment with light also has a stimulating effect on the subsequent chloride uptake (ARISZ 1947). During the pretreatment with light in a medium free from CO_2 a specific substance might have been formed (ARISZ and SOL 1956). This substance formed in the light is transportable. If an adjoining part of the exposed leafsegment remained in the dark during the pretreatment, it got part of the substance formed in the illuminated part, so that in this part, that was kept in the dark, the subsequent chloride uptake was higher as well. Both the uptake in the dark and the uptake in the light appeared to be stimulated by the substance formed in the light. A 150 f.c. preexposure gave a greater subsequent uptake both in the light and in the dark than a 50 f.c. preexposure. The light intensity therefore is important.

In the experiments made by ARISZ (1947), ARISZ and SOL (1956) the uptake was usually determined after a 24 hours absorption period by analysis of the material. In order to be able to check differences between the uptake of a series pretreated in the light and one in the dark, the time course of the uptake was here determined by the method described in Chapter 2.

LIGHT-DARK PRETREATMENT

How long can the substance formed in the light during the pretreatment affect the subsequent chloride uptake? In order to enable us to give an answer to this question a number of experiments were made, in which the rate of uptake of a series pretreated in the light was compared with that of a series pretreated in the dark. For this purpose two series were put in water in the 150 ml pretreatment vessels for 24 hours. One vessel was exposed to light, the other kept in the dark. The solutions were aerated with air free from carbon dioxide. After this pretreatment the series were transferred to the uptake vessels after which uptake took place from a solution of 0,001 M KCl + $CaSO_4$. Calcium sulphate was added to mitigate the toxicity of a one-salt solution. The results of these experiments showed that indeed there was a great difference between the rates of uptake of a series pretreated in the light and one pretreated in the dark. Moreover it appeared that the rate of uptake was rather variable in the course of time. This holds good both for a pretreatment in the light and one in the dark. We will revert to this later.

In a number of experiments the difference between the rates of uptake of the two series already diminished after six hours. This was usually due to the fact that the rate of uptake of the series pretreated in the light decreased more quickly than that of the other series. Figures 2 I and 2 II give a clear illustration of this phenomenon. After 12 hours the difference had disappeared (Fig. 2 II).

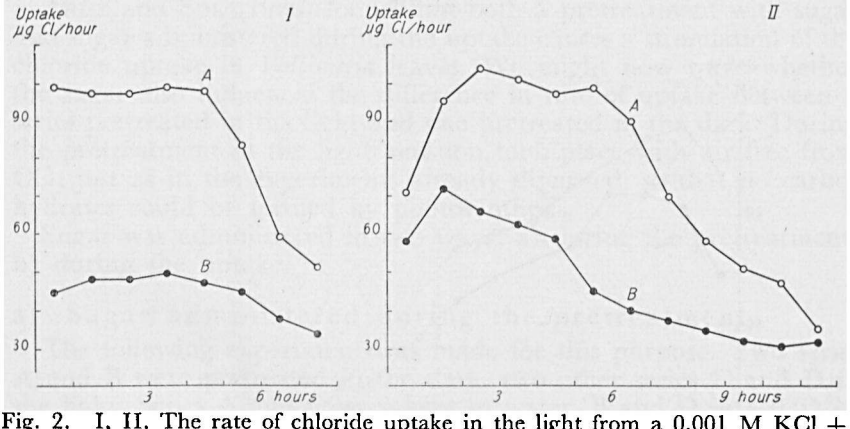


Fig. 2. I, II. The rate of chloride uptake in the light from a 0,001 M KCl + $CaSO_4$ solution after a pretreatment in water during 24 hours in the light (A) or in the dark (B).

A different picture gave an experiment, the result of which is represented in Fig. 3. In this case the difference in rate of uptake continued fairly constant during the first twelve hours. After this period they were transferred to 200 ml vessels containing a solution of the same composition, from which they could take up during the night. By doing so the composition of the solution, from which uptake

occurred during the night, changed to a slight degree only. Twenty four hours after the experiment had been started the rates of uptake of the two series were again determined, and the two series transferred to the uptake vessels. The difference in rate of uptake had disappeared by then. An identical picture is given by Fig. 4, where after 24 hours the difference in the rates of uptake of the two series had likewise become nil. In this experiment the influence of a pretreatment in the

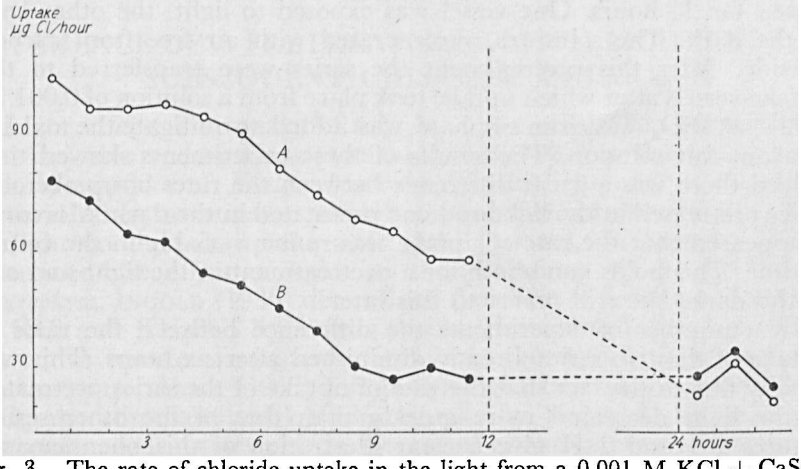


Fig. 3. The rate of chloride uptake in the light from a 0,001 M KCl + $CaSO_4$ solution after a pretreatment in water during 24 hours in the light (A) or in the dark (B).

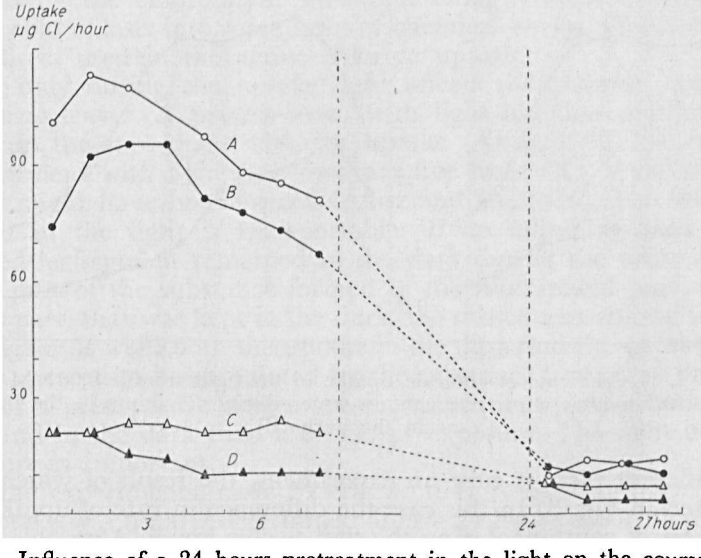


Fig. 4. Influence of a 24 hours pretreatment in the light on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution. A. Pretreatment light, uptake light. B. Pretreatment dark, uptake light. C. Pretreatment light, uptake dark. D. Pretreatment dark, uptake dark.

light on the subsequent uptake in the dark was also determined. It can be seen that a pretreatment in the light also influenced the uptake in the dark. The difference between the uptake in the light and the one in the dark had grown very small after 24 hours.

Besides it appeared from this experiment that illumination during the uptake stimulated the uptake more strongly than an exposure to light during the pretreatment. The difference in uptake of the series A and B gives an impression of the influence an exposure during the pretreatment had on the subsequent chloride uptake in the light. The difference in uptake of the series C and D gave this effect of pretreatment in the light on the subsequent uptake in the dark. On the other hand the effect of the light during the uptake appeared from the differences in uptake of the series A and C and that of the series B and D.

From the experiments, described above, we arrive at the conclusion that the influence of the specific substance formed during the pretreatment grew less and less during subsequent chloride uptake. Sometimes already after 12 hours', but certainly after 24 hours' uptake, its influence had disappeared. The substance formed during the pretreatment in the light not only stimulated the uptake in the light, but also the uptake in the dark. The latter is in accordance with ARISZ and SOL's (1956) experiments.

THE INFLUENCE OF SUGAR ON THE DIFFERENCE IN RATE OF UPTAKE OWING TO PRETREATMENT IN THE DARK OR IN THE LIGHT

ARISZ and SOL (1956) found that both a pretreatment with sugar and sugar administered during the uptake causes a stimulation of the chloride uptake in *Vallisneria* leaves. We might now trace whether the sugar also influences the difference in rate of uptake between a series pretreated in the light and one pretreated in the dark. During the pretreatment in the light aeration took place with air free from CO_2 just as in the experiments already discussed, so that no carbohydrates could be formed by photosynthesis.

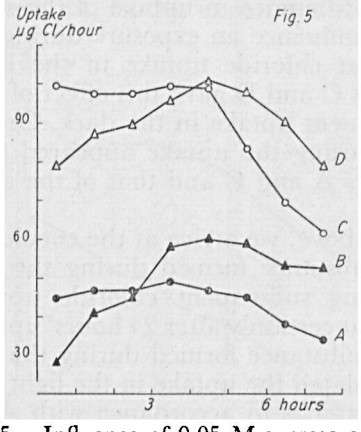
Sugar was administered in two ways: a) during the pretreatment, b) during the uptake.

a) Sugar administered during the pretreatment

The following experiment was made for this purpose. Two series A and B were pretreated in the dark, two other series C and D in the light. Series A and C were kept in water, B and D in a 0,05 M sucrose solution. In all series the uptake took place from a solution of 0,001 M KCl + $CaSO_4$ in the light.

As appears from Fig. 5: the course of the rates of uptake, was altered by the pretreatment with sugar. This result will be discussed in the next chapter in closer detail. If the difference in uptake between A and C (i.e. of the two series pretreated without sugar) is compared with the difference in uptake between B and D (the two series pretreated with sugar) it appears that in spite of the addition of sugar a great difference in rate of uptake continued to exist between the series

pretreated in the light and those pretreated in the dark. In fact the differences proved to be about equal as can be seen from Table I, in which the total uptake of the four series in 8 hours has been stated. The difference in total uptake of C and A 322 μg Cl and of D and B 315 μg Cl showed that the pretreatment has the same effect, in spite of sugar being added to D and B during the pretreatment. The diffe-



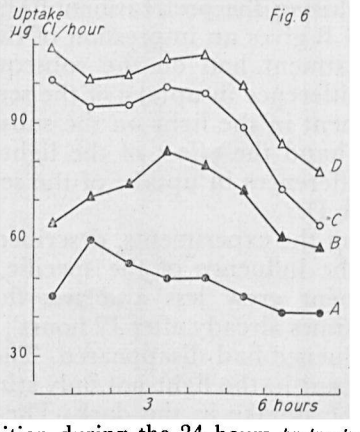


Fig. 5. Influence of 0,05 M sucrose addition during the 24 hours *pretreatment* in the light or in the dark. Uptake in the light from a 0,001 M KCl + $CaSO_4$ solution. A. Pretreatment dark. B. Pretreatment dark + sucrose. C. Pretreatment light. D. Pretreatment light + sucrose.

Fig. 6. Influence of 0,05 M sucrose addition during the *uptake* on the difference in the rate of uptake caused by a pretreatment in the light or in the dark. Uptake in the light from a 0,001 M KCl + $CaSO_4$ solution. A. Pretreatment dark, uptake — sucr. B. Pretreatment dark, uptake + sucr. C. Pretreatment light, uptake — sucr. D. Pretreatment light, uptake + sucr.

TABLE I

Influence of light and sucrose during the pretreatment on the course of the subsequent chloride uptake. Uptake from a 0,001 M KCl + $CaSO_4$ solution. Light intensity during the uptake and the pretreatment 100 f.c.

serie	pretreatment	total uptake 8 hours μg Cl.	influence of light
A	dark	357	C–A 322
B	dark + 0,05 M sucr.	398	D–B 315
			influence of sugar
C	light	679	B–A 41
D	light + 0,05 M sucr.	713	D–C 34

rence between D and C: 34 μg Cl gives the influence of sugar with the series pretreated in the light, the difference between B and A: 41 μg Cl the increase of the uptake owing to addition of sugar with the series pretreated in the dark. These results corroborated the results, obtained by ARISZ and SOL in 24 hours' experiments.

b) Sugar administered during the uptake

Two series A and B were pretreated in the dark, C and D in the light. A and C absorbed from a solution of 0,001 M KCl + $CaSO_4$

without addition of sugar, B and D from an identical solution to which 0,05 M sucrose had been added. Figure 6 and Table II give the result of this experiment. Owing to sugar addition during the uptake series B showed a higher uptake than series A, and series D a higher uptake than series C. On comparing these effects we see that after a pretreatment with light, addition of sugar during the uptake had less effect than after a pretreatment in the dark. This phenomenon was already observed by ARISZ and SOL (1956) in 24 hours' experiments. The total uptake of this series over an 8 hours' period are given in Table II

TABLE II

Influence of light during the pretreatment and of sucrose addition during the uptake on the course of the chloride uptake. Uptake from a 0,001 M KCl + $CaSO_4$ solution. Light intensity during the uptake and the pretreatment 100 f.c.

serie	pretreatment	uptake	total uptake 8 hours μg Cl.	influence of light
A	dark	light	396	C–A 317
B	dark	light + 0,05 M sucr.	562	D–B 225
				influence of sugar
C	light	light	713	B–A 166
D	light	light + 0,05 M sucr.	787	D–C 74

The difference in uptake between C and A 317 μg Cl and between D and B 225 μg Cl was due to the light during the pretreatment. The effect was distinctly greater than the effect of sugar addition during the uptake. From the difference between B and A 166 μg Cl and D and C 74 μg Cl we may conclude that the sugar had influenced the uptake of series B, which had been pretreated in the dark, more than the uptake of series D, which had been pretreated in the light. This may indicate that during the pre-exposure besides the "light-substance" other substances may be formed the effect of which can also be obtained by addition of sugar during the uptake period.

SHORT LIGHT-INDUCTION PERIOD WITHOUT CONCURRENT CHLORIDE UPTAKE

In the previous experiments the rate of uptake of a series pretreated with water in the light for 24 hours was compared with the rate of uptake of a series which had been pretreated in water in the dark for the same time in order to enable us to determine the effect of a pretreatment with light.

Owing to this pretreatment in the light a specific substance was assumed to be formed which increases the uptake of chloride ions. How fast is this substance formed? An attempt was made to give an answer to this question by carrying out experiments in which the series were allowed to absorb chloride for a couple of hours in order to determine the absorption capacity. Than one of the series was transferred to water in the light for a variable period in the absence of

carbon dioxide and the effect of this treatment on subsequent chloride uptake was determined.

We started with experiments in which after a two hours' uptake from the potassium chloride solution one of the series was transferred to water during the third hour. To a second series water was also administered but this series was darkened by wrapping the vessel in thick black paper. An other series served as a control. This series could take up chloride from the potassium chloride solution during the third hour. The rate of uptake during the fourth hour was in all three series equal to that of the first two hours. Therefore the one hours watertreatment in the light or in the dark has had no influence on the rate of the subsequent chloride uptake. Also the fourth series which got water in the light during the third and fourth hours, did not show a higher uptake after this light period than in the first two hours. So this period was apparently still too short to get an effect of the light. That is why in a subsequent experiment after a two hours' uptake from the potassium chloride solution an induction period of three hours was given. Here too the uptake of the series treated with water in the light or in the dark was equally great before and after this period (Fig. 7). The rates of uptake were indeed higher

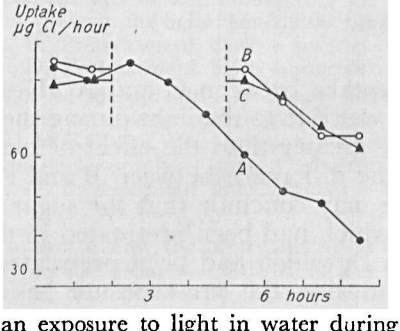


Fig. 7. Influence of an exposure to light in water during three hours between two uptake periods. Pretreatment 24 hours in water in the dark. After 2 hours uptake from a 0,001 M KCl + $CaSO_4$ solution series A remains in this solution exposed to light; series B is during the 3 hours in water in the light; series C 3 hours in water in the dark. In the third period all series in the light in KCl + $CaSO_4$ solution.

than those of the control series which had taken up chloride constantly, but the uptake of this series has decreased fairly rapidly in this period. The absorption curves of the other series had been shifted to the right owing to the water treatment. The total quantity of chloride which the three series had taken up after six hours absorption from potassium chloride solution was fairly equal.

Clearly, even a three hours induction period was too short to obtain an increased rate of uptake. It seemed possible that owing to the preceding uptake period, exposure to light during a water period did not show any influence, because the ions still present in the plasm counteracted this effect. In a subsequent experiment therefore the short light period was not introduced after a short uptake period, but

at once after the pretreatment. This might be called an additional pretreatment. Fig. 8 gives the result of this experiment, in which after a 24 hours' pretreatment in the dark one of the series received a 3 hours' additional pretreatment in the uptake vessel with water in the light. Each hour the water in the vessel was renewed. No chloride ions were released to the water. The rate of uptake of this series (B) is higher during the first two hours than that of the control series (A) in the first two hours. Here therefore we found a favourable effect of a three hours' pretreatment in the light and in the water.

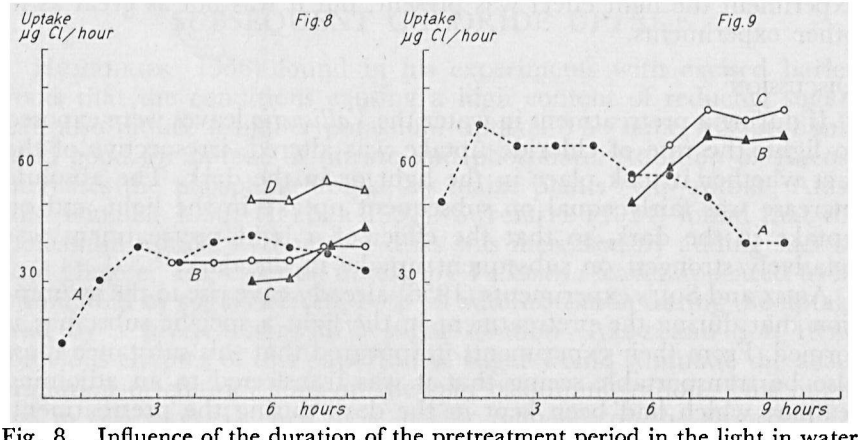


Fig. 8. Influence of the duration of the pretreatment period in the light in water on the subsequent chloride uptake. All series 24 hours pretreatment in the dark. Series A no additional pretreatment; series B 3 hours additional pretreatment in the light; series C 5 hours additional pretreatment in the dark; series D 5 hours additional pretreatment in the light. Uptake from a 0,001 M KCl + $CaSO_4$ solution.

Fig. 9. Influence of the duration of the pretreatment period in the light in water on the subsequent chloride uptake. All series 24 hours pretreatment in the dark. Series A no additional pretreatment; series B 5 hours additional pretreatment in the dark; series C 5 hours additional pretreatment in the light. Uptake from a KCl + $CaSO_4$ solution in the light.

A third series (D) received an additional pretreatment with water in the *light* for *5 hours*. The rate of uptake of this series is higher than that of the previous series. It was realized that these differences might also be based on a favourable effect of the extended water treatment. That is why a fourth series (C) received an additional pretreatment with water in the *dark* for *5 hours*. The rate of uptake of this series was for the first few hours averagely a little higher than that of the control series A. During this five hours' period in water in the dark hardly any change had therefore occurred in the absorption capacity. The uptake of this series was considerably lower than that of series D, which had been pretreated with water in the light for an additional five hours. This showed that light itself had increased the absorption capacity during the 5 hours treatment, presumably by the formation of a specific substance.

Besides one got the impression from this experiment that an addition-

al pretreatment in water altered the course of subsequent uptake, owing to the fact that under influence of the pretreatment the uptake was different from that of the control series mainly during the first few hours. The experiment of figure 9 is an instance of this. In this experiment the total uptake during a 6 hours' period of the control series (A) was 319 μg Cl, that of the series with 5 hours' additional water pretreatment in the dark (B) 324 μg Cl. So no difference with the control series. With a 5 hours' additional pretreatment in the light (C) the total uptake during 6 hours is 344 μg Cl. So, also in this experiment the light effect was present, but it was not as great as in other experiments.

Discussion

If during a pretreatment in water the *Vallisneria* leaves were exposed to light, the rate of chloride uptake was altered, irrespective of the fact whether it took place in the light or in the dark. The absolute increase was fairly equal on subsequent uptake in the light and on uptake in the dark, so that the effect of a light pretreatment was relatively strongest on subsequent uptake in the dark.

Arisz and Sol's experiments (1956) already gave rise to the assumption that during the pretreatment in the light a specific substance is formed. From their experiments it appeared that this substance must also be transportable seeing that it was transferred to an adjoining leafpart which had been kept in the dark during the pretreatment. If afterwards the exposed part was separated from the part which had been kept in the dark, both parts showed an increase of the subsequent chloride uptake.

The experiments described above show that the substance formed in the light continues to be active for a considerable time. After 24 hours the influence of the pretreatment is hardly perceptible any longer.

All experiments were made under conditions in which the carbon dioxide concentration was kept as low as possible by aeration with air free from CO_2, so that in the light hardly any carbohydrates could be formed.

Administration of sugar during the pretreatment influenced the time course of the uptake (Fig. 5). In spite of the addition of sugar there continued to be a great difference between the series pretreated in the light and the one pretreated in the dark. The effect of the specific substance produced during the pretreatment was not changed by it.

Addition of sugar during the uptake caused a distinct rise in the chloride uptake. The sugar effect was stronger after a pretreatment in the dark than after one in the light. Neither could sugar addition here equal the effect of a pretreatment in the light. The fact that sugar addition after a pretreatment in the dark had a stronger influence might indicate that in pretreatment in the light beside a specific substance already mentioned other substances had been formed as well, which may be substituted by substances formed in the presence of sugar.

A 3 to 5 hours' additional pretreatment in water in the light was sufficient to give a perceptible increase in chloride uptake. Light was essential in this, seeing a pretreatment in the dark had no effect. A 1 to 3 hours' exposure to light in water between two uptake periods had no demonstrable effect.

CHAPTER 4

INFLUENCE OF A PRETREATMENT WITH SUGAR ON THE SUBSEQUENT CHLORIDE UPTAKE

HUMPHRIES (1956) found in his experiments with excised barley roots that the conditions causing a high content of reducing sugars can also induce a higher potassium uptake. The same will probably hold good for uptake of nitrate and phosphate. Addition of glucose increases the phosphate uptake by maize plants (VAN ANDEL, ARISZ and HELDER 1950; HELDER 1952). SUTCLIFFE (1954) found that the potassium uptake by beetroot disks was increased by adding sucrose and glucose. The chloride uptake by *Vallisneria* leaves appeared to be stimulated by sugars as well, both on administration during the uptake and by a pretreatment in a sugar solution (ARISZ and SOL 1956; previous chapter of this paper). The sugar would stimulate the accumulation of chloride ions on the spot of administration. This might act in two ways; i.e. first by a specific influence on the absorption of ions from the outer solution into the symplasm, secondly by promoting the secretion process, when ions of the symplasm are accumulated in the vacuole.

In the experiments made by ARISZ and SOL (1956), the total uptake after 24 hours was determined by analysis of the plant material. By this method no impression was obtained of the influence of the sugar on the time course of uptake. Therefore these experiments have been repeated by the method described above. In Chapter 3 the fact was already mentioned that a pretreatment with sugar altered the shape of the curve representing the rates of uptake (Fig. 5). The series having had a pretreatment with sucrose, took up less the first few hours than the control series with a water pretreatment. This held good for a pretreatment in the light as well as in the dark. This inhibiting action had usually disappeared after three, four or five hours. After that the uptake of the series pretreated with sugar was greater. The rate of uptake of this series decreased less rapidly than the one of the series pretreated with water. This was especially perceptible in the experiments for which the rate of uptake had been determined for a longer period. Fig. 10 gives the result of an experiment, in which the rate of uptake of a series pretreated with water and of one pretreated with sucrose was determined for twelve hours. During the following night the series were transferred to larger vessels containing the same salt solutions from which they could absorb the next twelve hours. After this period they were transferred back to the small vessels and the rate of uptake was determined again. The rate of uptake of the series

pretreated with sugar decreased less rapidly than that of the other series. After 24 hours the rate of uptake of the two series was the same. That means that the influence of the sugar pretreatment had completely disappeared after 24 hours. We may conclude from the ex-

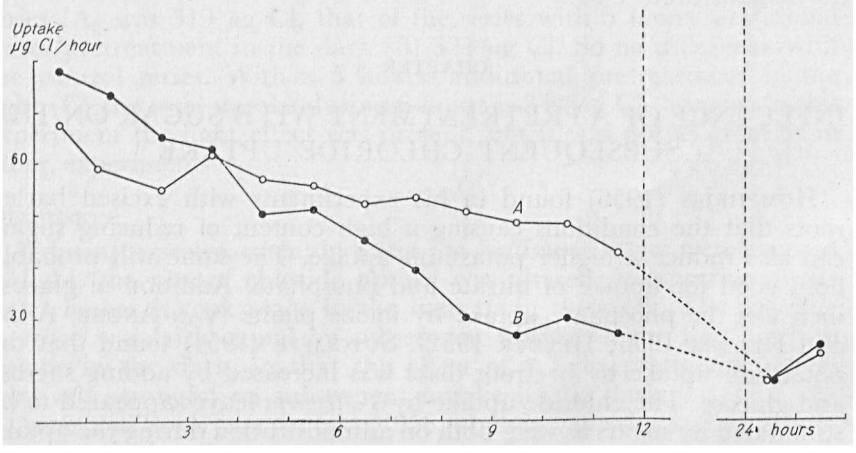


Fig. 10. Influence of a pretreatment with 0,05 M sucrose on the course of the subsequent chloride uptake. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Series A 24 hours pretreatment with sucrose; series B in water.

periments described here that the effect of the sugar pretreatment which were found in the twenty four hours' experiments by ARISZ and SOL, should be especially attributed to an increased uptake after the third, fourth or fifth hours.

The concentration of the sugar solution also appeared to be of importance for the effects mentioned. Fig. 11 shows that by a pre-

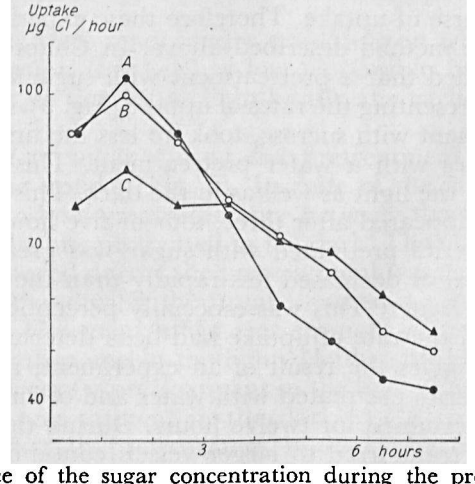


Fig. 11. Influence of the sugar concentration during the pretreatment on the course of the subsequent chloride uptake. Pretreatment during 24 hours in the dark: A water, B 0,025 M sucrose, C 0,05 M sucrose. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light.

treatment with 0,025 M sucrose no inhibition was caused during the first few hours. From the fifth hour, however, the uptake is greater. A pretreatment with 0,05 M sucrose caused a lower chloride uptake during the first three hours than the pretreatment with lower concentrations. The sixth, seventh and eighth hour the uptake was slightly greater than that of the series which received a pretreatment with 0,025 M sucrose. The difference, however, was hardly significant. Fig. 12 gives the result of an other experiment with various sugar concentrations. It appeared that in this experiment the inhibition

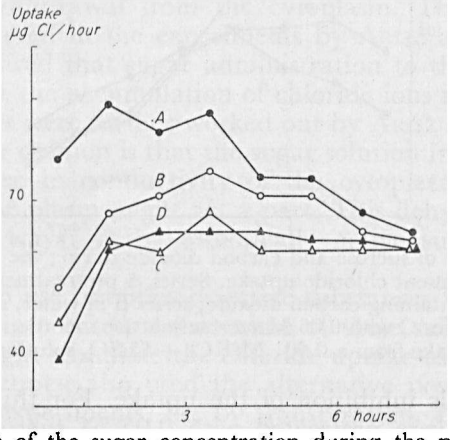


Fig. 12. Influence of the sugar concentration during the pretreatment on the course of the subsequent chloride uptake. Pretreatment 24 hours in the dark: A water, B 0,025 M sucrose, C 0,05 M sucrose, D 0,1 M sucrose. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light.

caused by the different sugar concentrations continued to exist for the entire eight hours' uptake period. The inhibition by 0,05 M and 0,1 M sucrose was equal, but greater than with a pretreatment with a 0,025 M solution. This latter solution therefore did cause a lower chloride uptake in this experiment. The difference was probably based on the difference in sugar condition of the material used for these two different experiments.

Have the products formed by photosynthesis the same effect as the sugar added? We have tried to find an answer to this question by pretreating in a following experiment a series (A) in water aerated with air, a second series (B) in water aerated with air free from CO_2 and a third series (C) in a 0,05 M sucrose solution aerated with air free from CO_2. We see from Fig. 13 that by a pretreatment with CO_2 the chloride uptake was greater the first few hours than of the series pretreated without CO_2, whereas the series pretreated with sugar, showed a lower uptake the first few hours. We may conclude from this, that in this respect there is a difference in action between the sugars possibly formed in the photosynthesis and the sugar solutions added from outside. The pH of the solution which was aerated with air containing CO_2 will have been slightly lower than of the two other series. This can, however, not explain the difference in uptake, just

because a pretreatment in solutions with a low pH causes a lower uptake, as will be discussed in Chapter 7.

A pretreatment with sugar decreased the rate of uptake during the first few hours. In a following experiment it was determined whether a short water treatment directly after the pretreatment with sugar

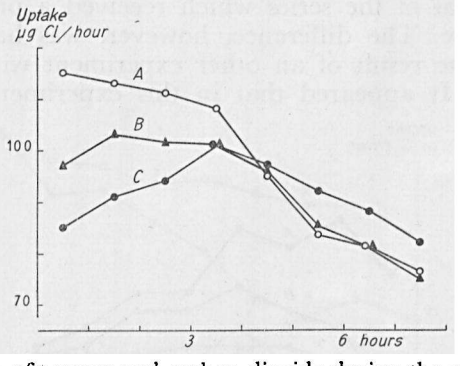


Fig. 13. Influence of sucrose and carbon dioxide during the pretreatment on the course of the subsequent chloride uptake. Series A pretreatment 24 hours in water aerated with air containing carbon dioxide; series B in water, aeration with carbon dioxide free air; series C with 0,05 M sucrose solution, aeration with carbon dioxide free air. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light.

could release this inhibition of the uptake. For this purpose two of the four series were pretreated with 0,05 M sucrose and two with water for 24 hours. Next the series were transferred to the uptake vessels. One series pretreated with sugar and one pretreated with water could take up chloride at once from the potassium chloride solution. The two other series first received an additional water treatment for an hour. This treatment appeared to have no effect on the chloride uptake. The inhibition of the uptake during the first few hours was not removed by this (Fig. 14 I, II). No stimulating effect of the sugar was found in this experiment.

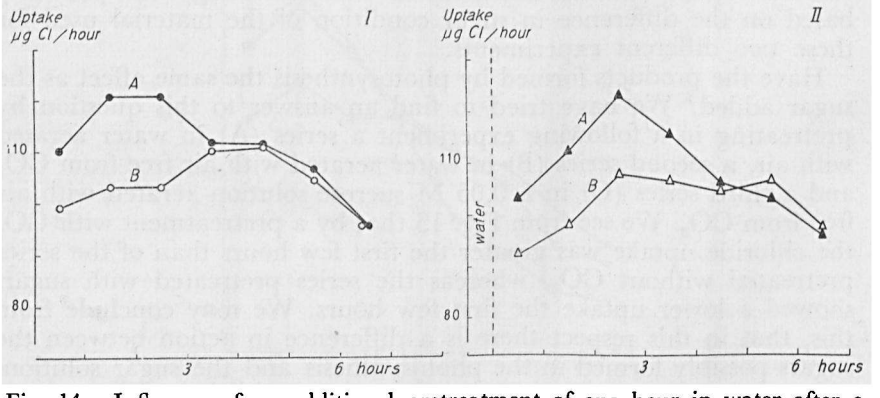


Fig. 14. Influence of an additional pretreatment of one hour in water after a pretreatment with 0,05 M sucrose. I. A pretreatment 24 hours in water; B pretreatment 24 hours with sucr. II. A pretreatment 25 hours in water; B pretreatment 24 hours with sucrose followed by a one hours pretreatment in water. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light.

Discussion

Sugar given during the pretreatment, was found to influence the subsequent chloride uptake in two ways.

1) An inhibiting action during the first few hours of the uptake period.
2) A stimulating influence after this initial period.

The inhibiting action might be an osmotic effect of the sugar. One of the possibilities is that the sugar acts on the structure of the plasm through water withdrawal from the cytoplasm. This phenomenon was already observed in the experiments by Arisz and Sol (1956), in which it appeared that sugar administration to the so called free part could inhibit the accumulation of chloride ions in the absorbing part. These results were further worked out by Arisz and Schreuder (1956 I, II) Their opinion is that the sugar solution in this case might act via a decrease in conductivity of the cytoplasm in which the dehydration of the plasm might act a part. This dehydration may be attained in two ways: 1) by osmotically active substances, 2) by transpiration.

If one wants to use osmotically active substances for dehydration, one should take into account that some of these substances may be absorbed and might stimulate the chloride uptake as well. Therefore Arisz and Schreuder also used the alternative possibility of dehydration in their experiments, i.e. by transpiration. In doing so they found that dehydration influences the accumulation and the transport of chloride ions.

Now the question is whether a dehydration during the pretreatment can also affect the subsequent rate of uptake of the chloride. It appeared from the following experiment (Fig. 15) that by giving a pretreatment in an atmosphere of a reduced vapour tension the subsequent chloride uptake decreased much. So the rate of uptake of the series (C) pretreated in an atmosphere in equilibrium with saturated Na_2SO_4 solution (relative vapour tension 93 % at 20° C) was lower than of the series (B) pretreated in an atmosphere in equilibrium with water. The uptake of this last series is again lower than that of series A pretreated in water. Sugar pretreatment gave quite a different shape to the curve (series D). This was due to the fact that we had to deal with two different effects of sugar on the uptake; 1) an osmotic effect which would act via the dehydration of the plasm, 2) a metabolic effect. The absorbed sugar might supply energy via metabolism for the accumulation of the chloride ions. These two effects may counteract each other. Initially in the uptake period dehydration of the plasm might have a stronger effect than the alteration of metabolism. Afterwards this effect decreases and the metabolic effect causes the rate of uptake to be greater than that of the control series.

Fig. 14 gives another indication that we have to deal with two processes. For here only the osmotic influence was present, no metabolic effect. The particularly high rates of uptake indicate that probably material was used containing many carbohydrates. Administration of

sugars during the pretreatment could have no effect any more in that case.

The inhibiting effect found here might be the same as SEEMANN (1953) found in leaves of *Salvinia natans* for waterpermeability. Glucose diminished the water permeability. He also thought that the sugar would act on the cytoplasm by a dehydration of the plasm.

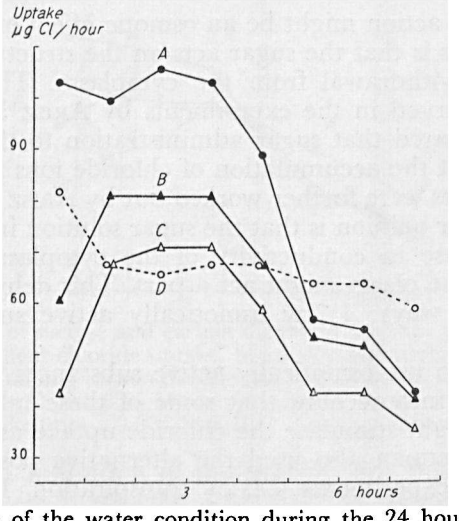


Fig. 15. Influence of the water condition during the 24 hours pretreatment in the dark on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Series A pretreatment in water; series B pretreatment in air in equilibrium with water; series C pretreatment in air in equilibrium with saturated Na_2SO_4 solution; series D pretreatment in 0,05 M sucrose solution.

KAHO (1956) investigated the influence of different sugars on the action of high concentrations of various salts on the plant plasm (beetroot and *Rhoeo discolor*), both during the pretreatment and the uptake. The sugars would decrease the permeability of the plasm for salts.

CHAPTER 5

INFLUENCE OF CARBON DIOXIDE ON THE CHLORIDE UPTAKE

In chapter 4 it was pointed out that sugars can stimulate the chloride uptake. The photosynthesis products too proved to be able to have a stimulating effect on the chloride uptake. For with a pretreatment with air containing CO_2 the chloride uptake was higher the first few hours (Fig. 1B). The effect of the CO_2, however, appeared to be dependent on the material used. This appears from the following experiments in which low salt as well as high salt material was used. The high salt material came from cultivation tanks filled with tapwater; the low salt material from tanks filled with deionised water,

the soil being poor in chloride. These experiments were made according to a method in which a 24 hours uptake period was used. The chloride uptake was determined of series of 8 leaflengths of 2.5 × 0,4 cms. After a 24 hours pretreatment in water the uptake took place from a solution of 0,001 M KCl + $CaSO_4$ for 24 hours. The solution was aerated with air or with air free from CO_2. The air containing CO_2 flew via a washing bottle with water to a closed uptake vessel. From the uptake vessel the air flew to a bottle containing barium hydroxide. The air free from CO_2 flew via a washing bottle with sodium hydroxide and next via a washing bottle with water to the uptake vessel and from the uptake vessel to a bottle containing barium hydroxide to show that the passing air was free from CO_2. In fact no precipitate was produced during the experiment. Before we started the experiments the solutions were aerated with air or air free from CO_2 for a considerable time. The chloride content of the material was determined by the VOLHARD method. By comparison with a control series the increase in chloride content could be determined.

In the way described above a number of experiments were made. The result has been stated in Table III. The uptake figures give the average of two replicates. In the case of low salt material there was no influence of carbon dioxide during the uptake. High salt material,

TABLE III

Influence of CO_2 addition during the uptake to low- and high salt material. Uptake during 24 hours from a 0,001 M KCl + $CaSO_4$ solution. Pretreatment and uptake in the light at 100 f.c.

exp.	material	uptake μg Cl/24 hours		pH at end exp.	
		+ CO_2	— CO_2	+ CO_2	— CO_2
54	low	518	510		
66	low	653	639	6,1	7,1
56	high	458	291	6,4	6,9
59	high	653	564	6,3	7,1

however, showed a much greater uptake with carbon dioxide. It might be imagined that by means of photosynthesis a product was formed stimulating the chloride uptake. The pH was measured at the end of the experimental period. The small pH differences, which were the same for high and low salt series, are not likely to have had an influence on the uptake in this experiment. If this had been the case the pH would have had a different influence on the uptake by low salt material and that by high salt material.

Is the difference in sugar content of the low- and the high salt material perhaps the cause of the phenomenon described above? High salt material is known to be often poor in sugars, whereas the low salt material would contain much sugar. In order to ascertain whether the sugar content of the material had any effect, two additional experiments with high salt material were made. Part of the material was pretreated with water, an other part with 0,02 M sucrose. The subsequent chloride uptake took place with or without CO_2 for 24

hours. The result of this experiment is recorded in Table IV. From this it appears that after a pretreatment of the high salt material with sugar there was not any difference between the uptake with or without CO_2.

TABLE IV

Influence of sucrose addition to high salt material during the pretreatment on the subsequent chloride uptake with or without CO_2. Uptake during 24 hours from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment during 24 hours in the light.

exp.	pretreatment	uptake μg Cl/24 hours	
		+ CO_2	— CO_2
62	water	423	360
	0,02 M sucr.	476	476
63	water	589	444
	0,02 M sucr.	603	596

From these experiments we therefore get the impression that CO_2 supply during the uptake has some influence only if the sugar content is low.

The question can also be raised whether in high salt material a pretreatment with CO_2 could have a similar influence on the difference in uptake with or without CO_2. The following experiment indeed pointed in this direction. Part of the material was pretreated with air containing CO_2, an other part with air free from CO_2. The uptake took place with or without CO_2 (Table V, exp. 73). It can be seen

TABLE V

Influence of CO_2 addition during the pretreatment and during the uptake on the chloride uptake from a 0,001 M KCl + $CaSO_4$ solution. Uptake and pretreatment in the light at 100 f.c.

pretreatment	uptake	uptake μg Cl/24 hours	
		exp. 73	exp. 96
+ CO_2	— CO_2	475	523
+ CO_2	+ CO_2	483	547
— CO_2	— CO_2	378	410
— CO_2	+ CO_2	483	403

that CO_2 during the uptake only showed some influence, if no CO_2 had been present during the pretreatment. In another experiment CO_2 had an influence during the pretreatment only (Table V, exp. 96). The difference with the preceding experiment was probably again due to differences in material.

DISCUSSION

From these experiments we gather the impression, that CO_2 supply during the uptake only had some effect in so called high salt material. VAN LOOKEREN CAMPAGNE (1957) found no influence of the carbon dioxide during the uptake. In view of the results presented here, it is likely that this difference in result must be ascribed to the longer

duration of the uptake and the different condition of the material. In particular, the quantity of sugar present in the material and the products formed from it, might play a considerable part in this. For by administering sugar during the pretreatment with material poor in sugar we got no effect of CO_2 during the uptake. By giving CO_2 during the pretreatment a similar effect could be obtained to that obtained on addition of sugar. Under these conditions the CO_2 during the uptake had no effect. So the products formed from CO_2 during the pretreatment had the same effect as the sugar added.

CHAPTER 6

INFLUENCE OF A PRETREATMENT WITH DIFFERENT SALT SOLUTIONS ON THE SUBSEQUENT CHLORIDE UPTAKE

According to some investigators a pretreatment with water has a favourable effect on the absorption capacity. STEWARD and PRESTON (1941) found this for potato disks. According to them this has to be attributed to the resumption of cell divisions and the accompanying protein metabolism.

REES (1949), SKELDING and REES (1952) thought of the removal of an inhibitor during the washing of beetroot disks. FAWZY, OVERSTREET, JACOBSON (1954) found for excised barley roots that an addition of different cations to solutions of different pH values during the pretreatment had an effect on the subsequent uptake. HELDER (1957) also found for young, growing barley plants a similar favourable effect of a waterperiod on the subsequent uptake of rubidium ions. Different salts appeared to be able to counteract this effect in a greater or less degree, either through an influence on the salt condition or through a more specific effect on the absorption mechanism.

In one of the previous chapters the formation of a specific substance during a pretreatment in light was discussed. Here the possibility was considered that the ions already absorbed might have an influence on the action of this substance. We may wonder what ions are responsible for this. In view of the results mentioned above it seemed useful to submit the influence of different ions administered during the pretreatment on the subsequent chloride uptake to a further investigation.

For this purpose a number of experiments were made in which the various series after a 24 hours pretreatment in water in the dark or in the light received an additional three hours pretreatment with a particular salt solution. Fig. 16 gives the result of an experiment in which four series A, B, C and D were pretreated in water in the dark for 24 hours. Next the series were transferred to the uptake vessels in the light. Series A received water, series B 0,0005 M $CaSO_4$, series C 0,0005 M K_2SO_4, series D 0,0005 M $CaCl_2$ for three hours. After this additional pretreatment the uptake per hour of chloride

from a solution of 0,001 M KCl + $CaSO_4$ for a period of 6 hours was determined for all series. It appeared that an additional 3 hours pretreatment with $CaSO_4$ did not influence the subsequent chloride uptake, for the rates of uptake of series A and B were equal. On our comparing the uptake of series C with that of series A, it appeared

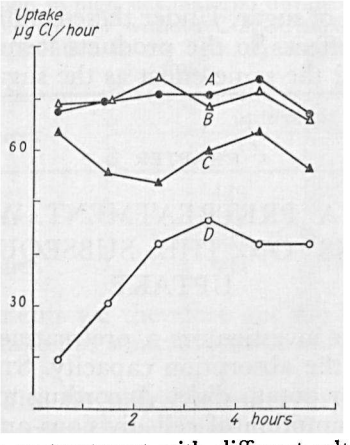


Fig. 16. Influence of a pretreatment with different salt solutions on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours additional pretreatment with different salt solutions in the light: series A water, series B 0,0005 M $CaSO_4$, series C 0,0005 M K_2SO_4, series D 0,0005 M $CaCl_2$.

that an additional pretreatment with K_2SO_4 diminished the chloride uptake. If we gave $CaCl_2$ this inhibition of the chloride uptake even appeared to be much stronger (D). The rate of uptake increased again after that. After 4 hours it had attained a more or less steady level. From the results of this experiment we may conclude that a particular combination of an anion and a cation during the pretreatment caused an inhibition of the uptake of chloride ions following this pretreatment. For Ca^{++} with SO_4'' caused no inhibition, K^+ with SO_4'' and Ca^{++} with Cl' did. It was possible that the chloride ions already absorbed from the $CaCl_2$ solution influenced the rate of uptake of the chloride ions. But the much lower uptake should also be attributed to a particular influence of the calcium ions during the pretreatment. This likewise appeared from the next experiment (Fig. 17), on the influence of various chlorides during the pretreatment. Series A had received an additional three hours pretreatment with water, series B could absorb from a KCl solution without calcium sulphate during these three hours, series C from a sodium chloride solution and series D from a calcium chloride solution. After this three hours period the chloride uptake from a potassium chloride solution, in which the usual quantity of $CaSO_4$ was present, was determined.

The chloride uptake during the pretreatment of series C from the sodium chloride solution was much lower than that of series B from the potassium chloride solution without calcium sulphate. During

the uptake from the potassium chloride solution with calcium sulphate following this three hours period it appeared, however, that the rate of uptake of series C was likewise lower than that of series B, the reverse being expected. So the chloride already absorbed during the pretreatment could not be the cause of the decreased uptake. The rate of

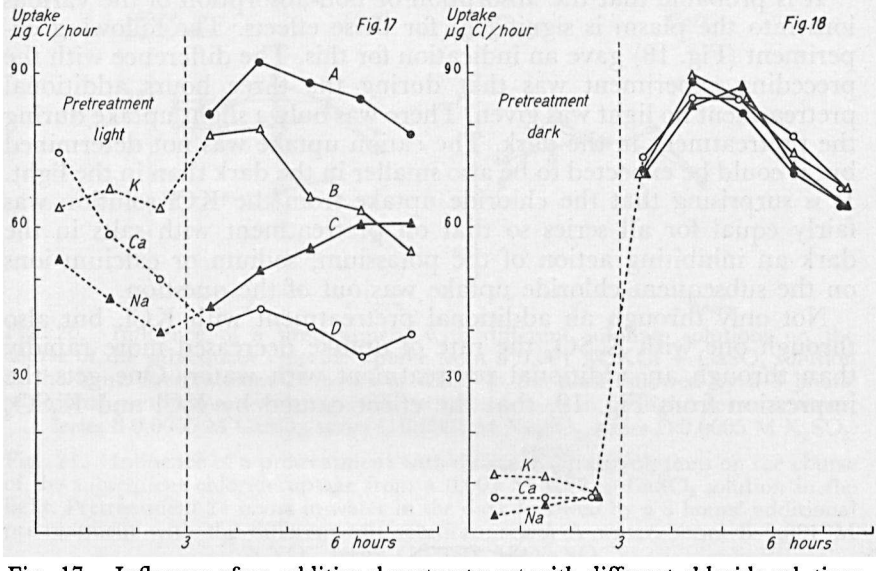


Fig. 17. Influence of an additional pretreatment with different chloride solutions in the *light* on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours additional pretreatment with the different salt solutions in the light: series A water, series B 0,001 M KCl, series C 0,001 M NaCl, series D 0,0005 M $CaCl_2$.

Fig. 18. Influence of an additional pretreatment with different chloride solutions in the *dark* on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours additional pretreatment with the different salt solutions in the dark: series A water, series B 0,001 M KCl, series C 0,001 M NaCl, series D 0,0005 M $CaCl_2$ (Symbols as in Fig. 17).

uptake of series C increased slowly. So we observed here the same phenomenon as was yielded by a pretreatment with calcium chloride in the previous experiment.

In this experiment too a pretreatment with $CaCl_2$ strongly inhibited the subsequent uptake from the KCl solution (series D). The rate of uptake, however, did not rise in this series. In all other experiments, however, we noticed an increase. Apart from this exception therefore we got the impression that the uptake capacity decreased under the influence of Ca and Na ions, but again increased after transfer to a potassium chloride solution.

Quite a different picture was given by series B which could first take up from a potassium chloride solution without calcium sulphate for three hours. On transfer to a solution of KCl + $CaSO_4$ the rate of uptake suddenly increased. After two hours, however, it again

decreased. So here we see a favourable effect of $CaSO_4$ during the uptake in the first place. We shall revert to this in the general discussion. On comparing series B with series A, we must infer that cations taken up during the pretreatment have an influence on the chloride uptake.

It is probable that the absorption or non-absorption of the various ions into the plasm is significant for these effects. The following experiment (Fig. 18) gave an indication for this. The difference with the preceding experiment was that during the three hours additional pretreatment no light was given. There was only a slight uptake during the pretreatment in the dark. The cation uptake was not determined but it could be expected to be also smaller in the dark than in the light. It is surprising that the chloride uptake from the KCl solution was fairly equal for all series so that on pretreatment with salts in the dark an inhibiting action of the potassium, sodium or calcium ions on the subsequent chloride uptake was out of the question.

Not only through an additional pretreatment with KCl, but also through one with K_2SO_4 the rate of uptake decreased more rapidly than through an additional pretreatment with water. One gets the impression from Fig. 19, that the effect caused by KCl and K_2SO_4

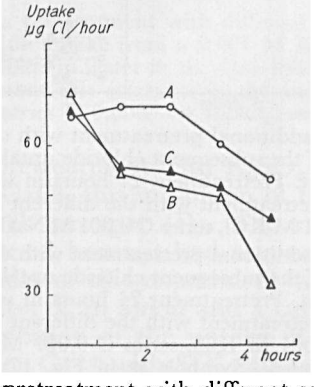


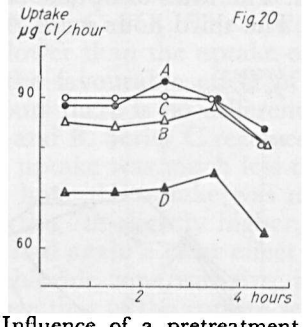
Fig. 19. Influence of a pretreatment with different salt solutions on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours' additional pretreatment with the different salt solutions in the light: series A water, series B 0,001 M KCl, series C 0,0005 M K_2SO_4.

was fairly identical. Also in the experiment of Fig. 20, K_2SO_4 brought about a strong inhibition of the uptake. Sodium sulphate, however, had no influence. The rate of uptake of the series additionally pretreated with $CaSO_4$ was slightly lower than that of the series pretreated with water. The difference, however, is hardly significant.

In a following experiment nitrate was used as anion (Fig. 21). It appeared that series B which had been in 0,001 M KNO_3 for 3 hours, took up as much chloride as series A which had had water for three hours. The uptake of the series which had had an additional pretreatment with $NaNO_3$ (series C) also appeared to be equal to that

of the control series. From these experiments it is therefore clear that the effect of the cation depended on the accompanying anion.

In these experiments material was used which was little sensitive to a light pretreatment. An additional pretreatment in water in the light for three hours with this material had no effect. This has been



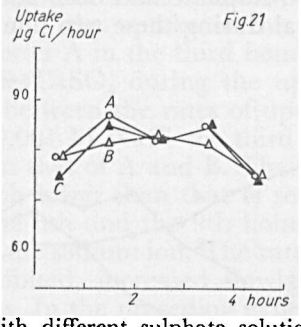


Fig. 20. Influence of a pretreatment with different sulphate solutions on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours' additional pretreatment with the different salt solutions in the light: series A water, series B 0,0005 M $CaSO_4$, series C 0,0005 M Na_2SO_4, series D 0,0005 M K_2SO_4.

Fig. 21. Influence of a pretreatment with different nitrate solutions on the course of the subsequent chloride uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Pretreatment 24 hours in water in the dark followed by a 3 hours' additional pretreatment with the different salt solutions: series A water, series B 0,001 M KNO_3, series C 0,001 M $NaNO_3$.

checked by comparing two series one of which had an additional pretreatment in water in the dark for three hours, the other an equal period in the light. The chloride uptake of the two series following this pretreatment was equal.

It was realized that the inhibiting effect found in the experiments mentioned above could also be ascribed, at least partly, to a stimulating effect of the additional watertreatment. From experiments already discussed before it, however, appeared that a three hours additional pretreatment with water usually had no effect.

However, in order to exclude the influence of these factors completely, a different method was used. By this the absorption capacity was determined first by putting the series in a potassium chloride solution for two hours, and then a different salt solution was given during the third hour, after which the uptake of chloride ions from a potassium chloride solution was determined again. In this way one could determine the influence of the action of the different ions for one hour on the subsequent chloride uptake.

From previous experiments it has appeared that calcium chloride gave a stronger inhibition than sodium chloride. That means that the combination of a bivalent cation with a monovalent anion gave a greater inhibition than that of a monovalent cation accompanied by a monovalent anion.

By the new procedure exactly the same result was obtained. More-

over it appears, that a trivalent cation in the presence of a monovalent anion gave an even stronger inhibition. The following experiment gave a clear picture of this (Fig. 22). After a twenty four hours pretreatment in the dark all series could first take up for two hours from a potassium chloride solution to which the above mentioned quantity of calcium sulphate had been added. The rates of uptake of all series were equal during these two hours. The third hour series A received

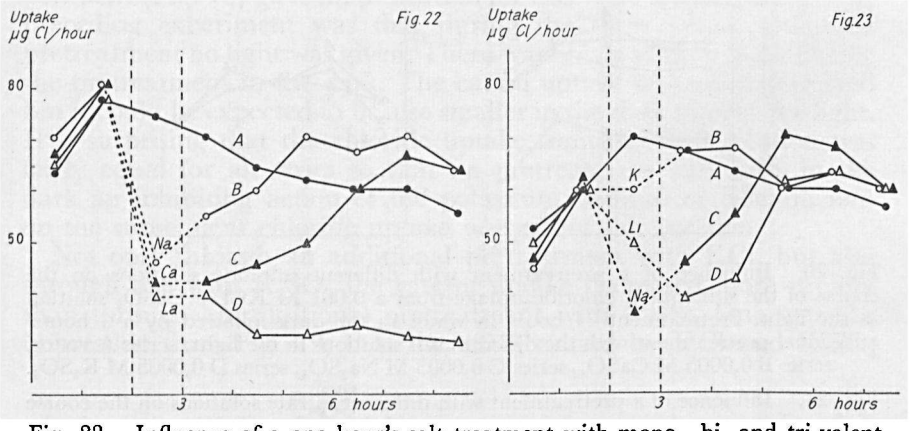


Fig. 22. Influence of a one hour's salt treatment with mono-, bi- and tri-valent cations on the course of the subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution. Only during the third hour uptake from the different chloride solutions: series A 0,001 M KCl + $CaSO_4$, series B 0,001 M NaCl, series C 0,0005 M $CaCl_2$, series D 0,0003 M $LaCl_3$.

Fig. 23. Influence of a one hour's salt treatment of different monovalent cations on the course of the subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Only during the third hour uptake from different salt solutions: series A 0,001 M KCl + $CaSO_4$, series B 0,001 M KCl, series C 0,001 M NaCl, series D 0,001 M LiCl.

the same solution, series B 0,001 M NaCl, series C 0,0005 M $CaCl_2$, series D 0,0003 M $LaCl_3$. So the chloride content of these solutions was identical. We now see that during the fourth hour series B took up less than series A, owing to the NaCl administered during the third hour. During the 5th hour the uptake was about equal, during the 6th, 7th, 8th and 9th hours, however, higher. Calcium chloride caused a stronger inhibition. During the 4th, 5th and 6th hours there was a considerable inhibition with respect to series A, during the 7th hour the uptake was equal and the 8th and 9th hours slightly higher. The lanthanum chloride caused a strong reduction in rate of uptake (D). We see in this case that the rate of uptake did not increase afterwards as it did in the series B and C. The third hour the chloride uptake of series B was greater than that of C, the uptake of C again greater than that of D. If the absorbed chloride ions alone had been responsible for the lower uptake after the third hour, it might be expected that the uptake of $B < C < D$. So if we compare the influence of NaCl, $CaCl_2$ and $LaCl_3$ with each other, it appears that

we have to deal with a cation influence in which the inhibition decreased in the order La > Ca > Na.

The effects of various monovalent cations were compared in a following experiment (Fig. 23). For the first two hours the four series A, B, C and D could take up from a potassium chloride solution to which $CaSO_4$ was added. The third hour series B received 0,001 M KCl without $CaSO_4$. The third hour the uptake of this series was slightly lower than the uptake of series A in the third hour. This was due to the favourable effect of the $CaSO_4$ during the uptake. The other hours there is no difference between the rates of uptake of the series A and B. Series C received 0,001 M NaCl the third hour. The chloride uptake was much less than that of A and B. The fourth and the fifth hour the uptake was much lower than that of series A, the 6th and the 7th slightly higher, the 8th and the 9th hour equal. So here we had again a clear effect of the sodium ion. The rate of uptake of the chloride ions was first inhibited, increased slowly and then rose above that of the control series .In the preceding experiment the same was found with respect to the influence of NaCl on the uptake.

The lithium ion caused an even stronger inhibition of the uptake than the sodium ion. Here too the rate of uptake rose till it had reached the rate of uptake of the control series. So it appears from this experiment that the influence of Li > Na > K. The inhibitions caused by the lithium and sodium ion proved to be reversible.

We have already seen that calcium chloride could have a considerable influence. Do other bivalent cations show a similar effect? In order to ascertain this the effect of $CaCl_2$, $MgCl_2$ and $SrCl_2$ was determined in the same way as in the previous experiment with monovalent cations. The different solutions were again given during the third hour. The effect on the subsequent chloride uptake was again determined. The inhibitions by $CaCl_2$ and $MgCl_2$ were equally great. (Fig. 24). $SrCl_2$ appeared to have a stronger effect. In all cases the inhibition was reduced in the course of time. So for bivalent

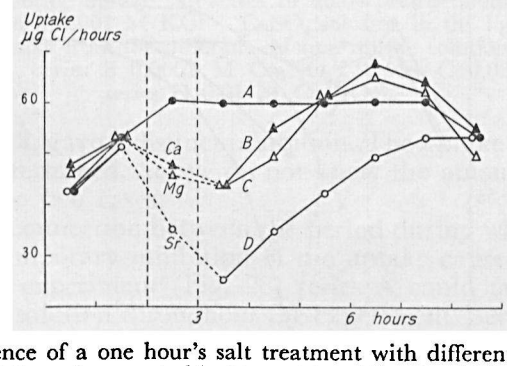


Fig. 24. Influence of a one hour's salt treatment with different bivalent cations on the course of the subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Only during the third hour uptake from the different *chloride* solutions: series A 0,001 M KCl + $CaSO_4$ solution, series B 0,0005 M $CaCl_2$, series C 0,0005 M $MgCl_2$, series D 0,0005 M $SrCl_2$.

cations an inhibiting influence was found which is almost entirely reversible.

In the following experiment it again appeared that also the anion was significant for the occurrence of these inhibiting effects. In this case the same bivalent cations were used, but instead of the chloride ion the nitrate ion. As shown in Fig. 25 $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$ caused no inhibition, as in the previous experiment the corresponding chlorides did.

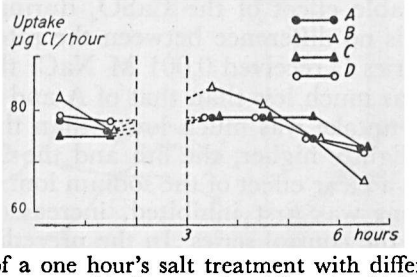


Fig. 25. Influence of a one hour's salt treatment with different bivalent cations on the course of subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Only during the third hour uptake from the different *nitrate* solutions: series A water, series B 0,0005 M $Ca(NO_3)_2$, series C 0,0005 M $Mg(NO_3)_2$, series D 0,0005 M $Sr(NO_3)_2$.

It was realized that the concentration used could be of importance for this inhibiting effect. That is why the influence of the concentration of various salt solutions on subsequent chloride uptake was further investigated. The inhibition by $CaCl_2$ appeared to be dependent on the concentration used. By our giving different concentrations of calcium chloride during the third hour, the strength of the inhibition of the chloride uptake in the subsequent hours was different. The four series A, B, C and D of Fig. 26 could first take up from a potassium

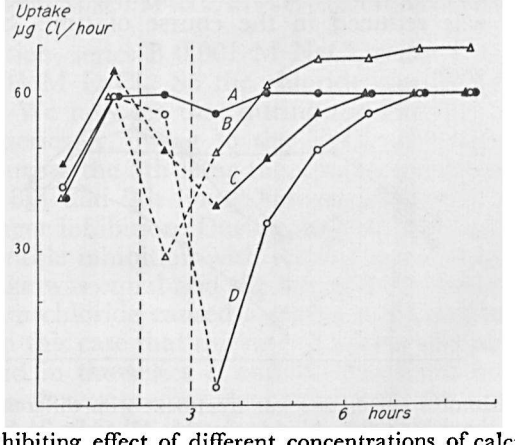


Fig. 26. The inhibiting effect of different concentrations of calcium chloride on the course of the subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Only during the third hour uptake from the different calcium chloride solutions: series A 0,001 M KCl + $CaSO_4$, series B 0,0001 M $CaCl_2$, series C 0,001 M $CaCl_2$, series D 0,01 M $CaCl_2$.

chloride with calcium sulphate solution for two hours. The third hour series A received 0,001 M KCl + 0,00027 M $CaSO_4$, series B only 0,0001 M $CaCl_2$, series C 0,001 M $CaCl_2$, series D 0,01 M $CaCl_2$. Series B showed a slight inhibition with regard to the control series A only the 4th hour, the 5th hour the uptake was equal, but the 6th, 7th, 8th and 9th hours there was an even higher uptake. The uptake of the 4th and 5th hours of series C was inhibited, the other hours it was equal to the control series. The inhibition, therefore lasted not only for a longer period than that of series B, but it was also much stronger. Series D gave an even stronger inhibition. The 4th hour the uptake was about nil, during the 5th, 6th and 7th hours it rose slowly till the rate of uptake of the control series had been reached. This experiment again gave a very clear picture of the change in the uptake mechanism caused by calcium ions. The uptake capacity, initially inhibited, increased slowly till a certain level had been reached.

In the previous experiments we found that $Ca(NO_3)_2$ 0,0005 M did not give an effect similar to that of $CaCl_2$ 0,0005 M. It is possible that the concentrations that cause inhibition are different for the two salts. That is why the effect of different concentrations $Ca(NO_3)_2$ were determined too. With the concentrations used, however, no effect was obtained (Fig. 27). In the preceding experiment the same con-

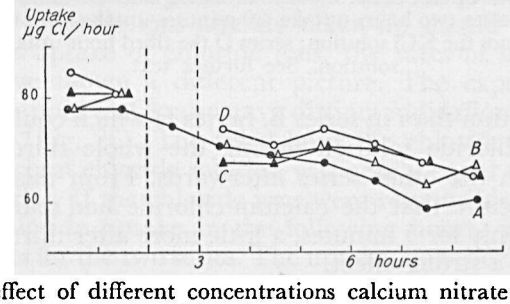


Fig. 27. The effect of different concentrations calcium nitrate on the course of the subsequent chloride uptake. All series 24 hours pretreatment in water in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution in the light. Only during the third hour uptake from the different calcium nitrate solutions: series A 0,001 M KCl + $CaSO_4$, series B 0,0001 M $Ca(NO_3)_2$, series C 0,001 M $Ca(NO_3)_2$, series D 0,01 M $Ca(NO_3)_2$.

centrations $CaCl_2$ gave a distinct inhibition. The uptake of the calcium ion was not determined. So we do not know the amounts of calcium taken up in the two cases.

Is there any connection between the period during which the $CaCl_2$ acts and the temporary inhibition of the uptake caused by this salt? In a following experiment (Fig. 28) series A could take up from a KCl + $CaSO_4$ solution throughout the experiment. Series B first took up from this solution for two hours, then 5 minutes from a calcium chloride solution followed by 55 minutes 0,001 M KCl + $CaSO_4$. The chloride uptake of series B from the potassium chloride solution during the third hour was computed from the period of 55 minutes. The chloride uptake in the third hour thus calculated was slightly

lower than that of the control series A. For the rest the rates of uptake of this series were equal to those of series A. Series C could take up from a calcium chloride solution during the first half of the third hour and during the latter half again from a KCl + $CaSO_4$ solution. The longer duration of the action of the calcium chloride gave as lightly

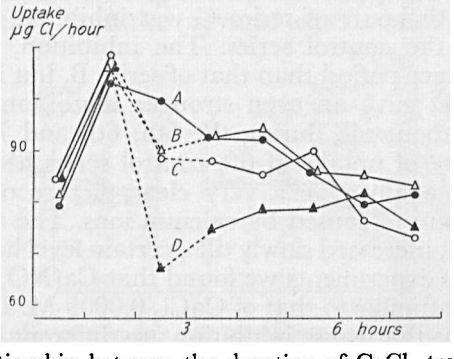


Fig. 28. The relationship between the duration of $CaCl_2$ treatment period and the subsequent chloride uptake from KCl solution. All series 24 hours pretreatment in the dark. Series A, controlseries, uptake during the whole experiment from a 0,001 M KCl + $CaSO_4$ solution; series B after two hours uptake from the KCl solution, 5 minutes uptake from 0,0005 M $CaCl_2$ and 55 minutes from the KCl solution; series C after two hours uptake, 30 minutes uptake from the $CaCl_2$ solution and 30 minutes from the KCl solution; series D the third hour uptake from the $CaCl_2$ solution. See further text.

stronger inhibition than in series B. Series D which could take up from the calcium chloride solution during the whole third hour took up much less than the other series afterwards. From this experiment it was clear therefore that the calcium chloride had scarcely any effect if it could act only for 5 minutes, a little more after thirty minutes, but after one hour a strong effect.

Two hours calcium chloride gave a still stronger inhibition than one hour. In Fig. 29 series B had received $CaCl_2$ during the third hour,

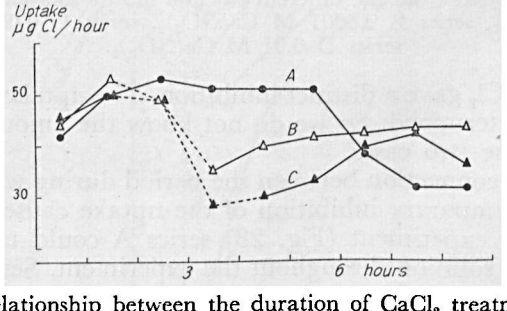


Fig. 29. The relationship between the duration of $CaCl_2$ treatment period and the subsequent chloride uptake from a KCl solution. All series 24 hours pretreatment in the dark. Series A, control series, uptake from a 0,001 M KCl + $CaSO_4$ solution during the whole experiment; series B during the third hour uptake from 0,0005 M $CaCl_2$ solution; series C during the third and during the fourth hour uptake from the $CaCl_2$ solution.

series C during the third and fourth hours. After the treatment with calcium chloride the rates of uptake of C were lower than those of B. So also in this experiment the inhibition caused by the calcium chloride was dependent upon the time during which it could act.

Discussion

The results of experiments described in this chapter indicate that it is possible to influence the subsequent chloride uptake by a pretreatment with different salt solutions. By an additional three hours pretreatment with various salt solutions the uptake of chloride ions was inhibited in some cases, in other cases it was not. The same was found for a treatment with various salt solutions for one hour after an KCl uptake period of a few hours.

The following salts had an inhibitory influence: NaCl, LiCl, $CaCl_2$, $MgCl_2$, $SrCl_2$, $LaCl_3$, K_2SO_4. Other salts such as KNO_3, $NaNO_3$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, $CaSO_4$ and Na_2SO_4 had no influence. Thus, the chloride uptake was inhibited if during the pretreatment a particular combination of an anion and a cation was applied.

The experiments indicated a specific cation influence. This appeared i.a. from the experiment with different chlorides (Fig. 17). During the pretreatment the chloride uptake from the various solutions was not the same. If the chloride ions already taken up should have had an influence on the uptake of chloride ions, the rates of uptake of the series would have shown a different picture. The experiment with various concentrations $CaCl_2$ also gave distinct indications of a specific cation influence (Fig. 26). The third hour the chloride uptake from the strongest calcium chloride solution was nearly equal to the uptake of the control series. If the chloride ions were responsible for the inhibition of the chloride uptake in the following hours, we ought not to find differences for the two series. The uptake, however, was strongly inhibited by the treatment with calcium chloride.

A pretreatment with K_2SO_4 or with KCl (without $CaSO_4$) had a similar effect (Fig. 19). Though during the pretreatment one series could take up chloride, the inhibition of the chloride uptake caused by these salts was fairly equal. It might be concluded from this experiment, that the chloride ions already taken up during the pretreatment had no influence on the rate of chloride uptake during the subsequent hours. The lower uptake would then have been caused by the K-ions. This experiment did not provide a sufficient proof of this. The other series could take up SO_4 ions during the additional pretreatment. If these SO_4 ions had been bound by the same sites as the chloride ions, they might also have influenced the chloride uptake in the same way.

In this connection we may point out that Leggett and Epstein (1956) found for barley roots that for P, NO_3 and Cl there is no measurable affinity for the sulphate-selenate binding sites.

Though the experiments indicated a specific cation influence there must have been a certain influence of the anion as well. In this connec-

tion the anions may have been significant through their influence on the absorption or non-absorption of the cations.

It is known that nitrates are only taken up by *Vallisneria* leaves under particular circumstances, i.e. at a high pH and in the presence of CO_2 (ARISZ, unpublished results). In the experiments described in this chapter, there could be only a slight uptake, if any, of nitrates, because they were made at a pH 5–6, the presence of carbon dioxide being prevented as much as possible.

By a pretreatment with potassium, sodium-, or calciumnitrate the chloride uptake was not inhibited in our experiments. So it might be imagined that the cations penetrate into the cell with difficulty in these cases, if there is a certain relation between the uptake of the cations and the anions. The same is likely to hold good for calcium sulphate.

It was strange that a pretreatment with Na_2SO_4 had no influence on the chloride uptake whereas K_2SO_4 treatment had. It is not clear what this difference was due to.

Light appeared to be an important factor for the occurrence of these inhibition phenomena. Here too the penetration of the cations into the plasm was probably significant. By a pretreatment with particular salts in the light the subsequent chloride uptake was inhibited. A pretreatment with the same salts in the dark had not any influence on the uptake of chloride ions (Fig. 18). The chloride uptake was very slight in the dark. The cation uptake was not determined. This will have been slight too, for WINTER (personal communication) found that the uptake of Rb by *Vallisneria* leaves in the dark was considerably lower than in the light.

The nature of the cation appeared to be significant for the inhibiting influence on the chloride ions uptake. So the inhibition caused by a trivalent cation appeared to be stronger than the inhibition by a bivalent cation. The bivalent cation had a stronger effect than a monovalent cation. The strength of the inhibition decreased in the following sequence $La > Ca > Na$. For monovalent and bivalent cations similar sequences were found, i.e. $Li > Na > K$ and $Sr > Mg = Ca$.

The inhibition caused by these cations was entirely or partly reversible. Only lanthanum chloride caused an irreversible inhibition. In this case the rate of uptake during the other hours of the uptake period remained at the same level, which was much lower than that of the control series.

From the experiments with different concentrations of $CaCl_2$ it appeared that the inhibition became stronger according as the concentration increased (Fig. 26).

The time during which the calcium chloride could penetrate was significant too. A 5 minutes treatment with $CaCl_2$ had hardley any effect, a treatment for 30, 60 and 120 minutes, however, had. According as the calcium chloride could act longer as the inhibition increased (Figs 28, 29).

CHAPTER 7

INFLUENCE OF THE pH DURING THE PRETREATMENT ON THE SUBSEQUENT CHLORIDE UPTAKE

The acidity of the different solutions used in the experiments described in the previous chapter was always about the same. So the different effects could not be attributed to it. The concentration of the H-ions of the solution during the pretreatment appeared to influence the subsequent uptake of chloride ions. In these experiments eight series were used at the same time. All series were first pretreated in water for 24 hours. Four of these series received an additional pretreatment for an hour in water that had been brought to various pH values with the aid of H_2SO_4. During this hour the four other series received a similar pretreatment. However, 0,0005 M $CaSO_4$ was added to the solutions of various pH. Fig. 30 shows that an addition-

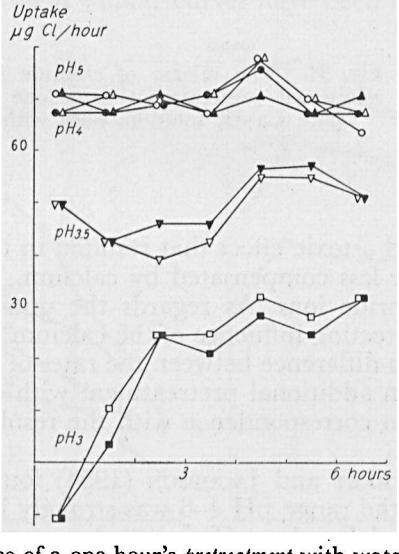


Fig. 30. The influence of a one hour's *pretreatment* with water of different pH and the addition of 0,0005 M $CaSO_4$ during this additional pretreatment. All series 24 hours pretreatment in the dark. Uptake from a 0,001 M KCl + $CaSO_4$ solution at pH 5,5. The open symbols with $CaSO_4$.

al one hour pretreatment in solutions of different pH influenced the subsequent chloride uptake. Between a pretreatment at pH 5,5 and pH 4 there was no difference. After a pretreatment in a solution of pH 3,5 the uptake was, however, much lower. This difference in rate of uptake continued to exist during the uptake period. The solution with a pH 3 appeared to have a very strong influence. During the first hour chloride ions were even released to the potassium chloride. After that the uptake increased slowly, but it continued to be much lower than that of the other series. It appeared that these effects

caused by the different pH's were not influenced by the presence or absence of calcium sulphate.

The loss of chloride ions during the additional pretreatment was also determined. It appeared that no chloride ions were released to the solutions of a pH 5.5, 4, 3.5. This held good for the water series as well as for the solutions containing calcium sulphate. To water that had been brought to a pH 3, however, many chloride ions were released. To the calcium sulphate solution of the same pH nearly half the quantity less (Fig. 31).

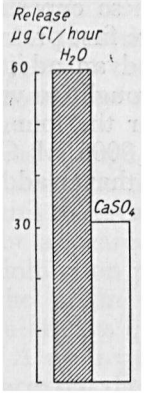


Fig. 31. The release of chloride ions during the additional pretreatment from one hour to water and $CaSO_4$ solution, both with a pH 3,0.

Discussion

The low pH had a toxic effect that resulted in the loss of salt. This effect was more or less compensated by calcium. This was expressed in the loss of chloride ions. As regards the uptake mechanism, no indication of a protecting influence of the calcium sulphate was found here. There was no difference between the rates of uptake of the series which have had an additional pretreatment with or without calcium sulphate. This is in correspondence with the results described in the previous chapter.

Fawzy, Overstreet and Jacobson (1954) found that the loss of potassium ions in the range pH 4–6 was strongly influenced by small concentrations of calcium ions. Owing to the presence of calcium ions much fewer potassium ions were released. They also speak of a protective effect of the calcium ions. A potassium uptake following this pretreatment was also influenced by the calcium ions. At the lower pH's the potassium uptake of the barley roots which have had a pretreatment with calcium chloride was much higher than that of the series pretreated with water (i.c. diluted hydrochloric acid). This influence of the calcium present during the pretreatment was not found in our experiments for the uptake of chloride ions. The uptake of potassium was not determined so that we cannot tell whether the effect of the calcium on the anion uptake and the cation uptake was different.

CHAPTER 8

GENERAL DISCUSSION

The results of the previous research show that the rate of uptake of chloride ions by *Vallisneria* leaves may be influenced by various pretreatments of the material for a shorter or longer period.

By the method described in Chapter 2 the course of the rates of uptake during a certain period could be accurately determined.

The lines representing the rates of uptake can deviate very much from each other in shape. The nature of the material used and the previous development is likely to be significant in this. Fig. 32 I gives a picture of the various shapes of the curves found in the experiments in which the material was pretreated in water in the dark for 24 hours. Shape C was of most frequent occurrence. Only in a few cases did a continuous decrease in rate of uptake occur (B). With a pretreatment in the light similar curves have been found (Fig. 32 II).

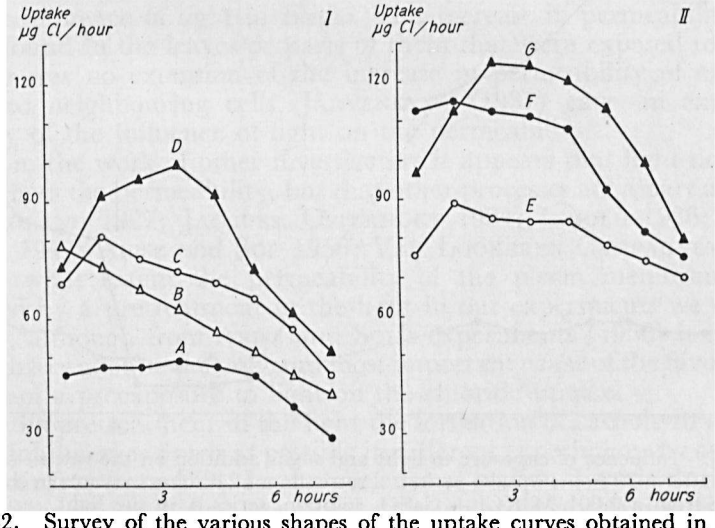


Fig. 32. Survey of the various shapes of the uptake curves obtained in the experiments in which the material received a 24 hours pretreatment in the dark (32 I) or in the light (32 II).

In some cases a shorter or longer induction appears to be needed before the maximum rate of uptake is reached (C and D Fig. 32 I; E and G Fig. 32 II). After some time the rate of uptake in nearly all experiments begins to decrease. To what this decrease is due, we do not know. There is a possibility that after an uptake period there arises in the cell a deficiency of a substance that is necessary to have the active chloride uptake progress at this high level in constant light. This substance might be of importance for the conversion of light energy to chemical energy, so that the energy needed for the active chloride uptake can no longer be formed. In spite of the exposure the rate

of uptake continues decreasing to a certain value, after which it remains constant for some time. The following preliminary experiment might give an illustration of this (Fig. 33). In this experiment series A could absorb from a potassium chloride solution for 28 hours, after a twenty four hours pretreatment in water in the light. After 24 hours the rate of uptake has become fairly equal to that of series B which had the same pretreatment, but in which the uptake occurred in the dark. So the light has almost ceased to influence the rate of uptake. After the 28th hour series A could take up from a potassium chloride solution of the same concentration to which 0,05 M sucrose had been added. The rate of uptake rises through this addition of sugar. This suggests that on our adding the energy needed for the active uptake in a different form, the material can take chloride at a higher rate. After the 28th hour series B received light during the uptake in the

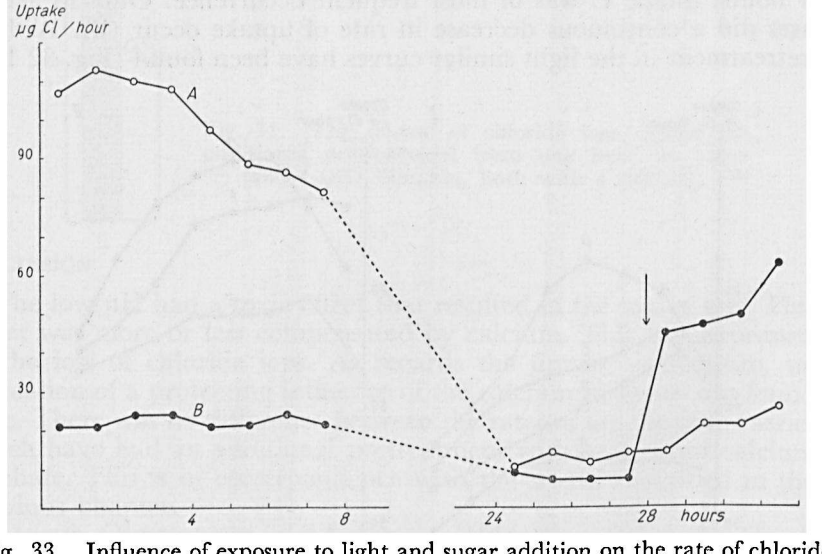


Fig. 33. Influence of exposure to light and sugar addition on the rate of chloride uptake after a 28 hours uptake period. Pretreatment 24 hours in water in the light. Uptake from a 0,001 M KCl + $CaSO_4$ solution, series A in the light, series B in the dark. From ↓ sugar addition to series A and also series B exposed to light.

absence of CO_2. This caused an immediate increase of the chloride uptake. During the preceding 28 hours this series had taken up much less chloride than series A, so that in series B there will not have been a deficiency of the substances needed for the conversion of light energy into another form available for active ion absorption.

Through a pretreatment in water in the light the subsequent chloride uptake is raised. It is likely that during this pretreatment a specific substance is formed which according to ARISZ and SOL (1956) is transportable. About the nature of this specific substance still little is known. But it does appear from this investigation that this substance formed during a 24 hours pretreatment continues to operate for at most 24 hours. Sometimes its influence has already disappeared after

12 hours. During a pretreatment of 3–5 hours in water in the light a sufficient amount of specific substance is formed to yield a noticeable effect. An exposure to light in water for 3 hours between two uptake periods on the contrary has no demonstrable effect. It might be that in this latter case the ions already taken up into the plasm can inhibit the formation of the specific substance. The experiments do not offer sufficient data to demonstrate the correctness of this supposition.

The influence of light during the uptake is much greater than that of a pre-exposure (Fig. 4). VAN LOOKEREN CAMPAGNE (1957) found no indications in his experiments of a specific substance being formed in the light during the uptake. Neither have any indications been found in this investigation for the formation of a specific substance during the uptake in the light. It is possible that in this case all light energy is immediately used for the uptake of chloride ions, whereas during a pretreatment with light in water the light energy can be used for the formation of a specific substance.

Light can also influence the permeability of the plasm. Thus LEPESCHKIN (1930) found an increase of permeability for aniline dyes under influence of light in *Elodea*. This increase in permeability was only found in the leaves or parts of them that were exposed to light. There was no extension of the increase in permeability of exposed cells to neighbouring cells. JÄRVENKYLÄ (1937) gave an extensive survey of the influence of light on the permeability.

From the work of other investigators it appears that light not only influences the permeability, but that other processes act a part as well. (HOAGLAND 1927; JAQCUES, OSTERHOUT 1934; INGOLD 1936; ARISZ 1943, 1947; ARISZ and SOL 1956; VAN LOOKEREN CAMPAGNE 1957).

To what extent the permeability of the plasm membrane was altered by a pretreatment in the light in our experiments we do not know, although from ARISZ and SOL's experiments (1956) it is clear that this cannot be the only and most important cause of the favourable effect of a preexposure to light on the chloride uptake.

In the pretreatment in the light the formation of carbohydrates has been inhibited as much as possible in different experiments by aeration with air free from CO_2. In the presence of carbon dioxide during the pretreatment or during the uptake the chloride absorption is sometimes stimulated. This appears to be dependent on the quantity of sugar present in the material or on the products formed from this sugar (Chapter 5).

Not only do the carbohydrates formed in the photosynthesis stimulate the chloride uptake, but also the addition of carbohydrates in the form of sucrose gives an increase in rate of uptake. It appears that this sugar has two effects, i.e. a metabolic effect and an osmotic influence which would act via the dehydration of the plasm (discussion Chapter 4). By the latter influence the conductivity of the plasm would be altered, which would cause the chloride uptake to be inhibited the first few hours. BROUWER (1954) also found a decrease in conductivity for water and anions for the roots of *Vicia faba* through addition of sugar to the medium in which the roots were kept.

Through a pretreatment with different salt solutions the uptake of chloride ions was inhibited in some cases, in others not. Though the experiments point to a specific cation influence, yet there must be a certain influence of the accompanying anion as well (discussion Chapter 6). After the treatment with the different salt solutions the chloride uptake again increases slowly in most cases after some time. This means that the blocking of the uptake mechanism caused by the various ions is slowly being released again.

The uptake mechanism is situated in the plasm. It is therefore obvious that for an explanation of the facts mentioned above the influence of the different ions on the plasm has to be taken into consideration. The cations might alter something in the plasm which causes some inhibition of the uptake of chloride ions. We might say the permeability for the chloride ions is strongly decreased.

On the permeability of salts many researches are known. In most older researches on the passive movement of salts the plasmolytic method was used. This method shows the entrance of salts into the vacuoles. We might speak here of transmeability (ARISZ 1945), in contrast to intrability (HÖFLER 1932, 1951), by which the uptake of substances from the medium into the plasm is meant. A survey of the older researches on the influence of salts on the transmeability is given by GELHORN (1929), GELHORN and REGNIER (1936). Among the older researches especially those of OSTERHOUT (1912, 1913) are important, because he was the first in pointing out the antagonistic action of salts. By determination of the conductivity BROOKS (1917) found that NaCl enters more rapidly through a Laminaria membrane than $CaCl_2$. Especially during the first few hours calciumchloride would cause a decrease in permeability. OSTERHOUT (1922) too found for *Laminaria* by conductivity measurements that calcium can inhibit the increase in permeability caused by alkali salts. A similar antagonism between calcium and alkali salts was also found by HÖFLER (1940), in his experiments on the influence of salts on the swelling of the cytoplasm. RABER (1921, 1923) showed that also the anions influence the permeability. KAHO (1924, 1926, 1933) likewise investigated the effect of salts on the plant plasm. According to him the penetration of salts is a physical chemical process dependent on the colloid activity of the salts in which both anion and cation are significant. The lipids in the surface layers would be of great significance here. An objection to KAHO's experiments is that he uses high concentrations which consequently have a toxic effect.

HANSTEEN CRANNER (1914, 1919) thinks that the toxicity of the salts is not caused within the cell, but by influencing the outer boundary surface of the plasm with the many phosphatide systems.

The sequence of the salts the various investigators give for their influence on the transmeability differ a good deal. This is due to the use of different material and to operating with different concentrations. Moreover, their experiments are concerned with a passive permeation of the salts into the vacuole, whereas in our experiments the influence of various salts on the active uptake of chloride into the plasm was

examined. In doing so it appeared that only by a pretreatment with various salts in the light the chloride uptake is influenced. This may indicate that we have to deal here with an active uptake of these salts into the plasm. An advantage of the method of investigation used here is that the various salts are given during a pretreatment, so that a competition between the different ions during the uptake is prevented. According to EPSTEIN and collaborators (1952, 1953, 1954) the inorganic ions combine with so called carriers. The complex formed in this way passes the boundary surface. Through a chemical conversion at the inside of the boundary surface it is liberated again. It now appears from the experiments of these investigators that different inorganic ions are bound by the same sites, so that the uptake of one ion is influenced by the other.

DE HAAN (1933, 1935) investigated the influence of salts on the waterpermeability for cells of the inner epidermis of bulbscales of *Allium cepa*. The cations diminish the waterpermeability in certain low concentrations, whereas they raise it at higher concentrations. LOEVEN (1951), SEEMANN (1953), VREUGDENHIL (1957) corroborate these results. DE HAAN, LOEVEN and VREUGDENHIL try to explain the results with the aid of the complex theory of BUNGENBERG DE JONG.

BUNGENBERG DE JONG and his collaborators tried to get an insight into the structure of the cellmembrane at the hand of artificial models. The models developed by them all have a common feature. A film of phosphatides constitutes the lipid fraction of the membrane. These phosphatides are cemented together with cholesterol. The negative charge of the membrane must be ascribed to a small amount of phosphatidic acid. Such a film would still be unstable but it may form a tricomplex with proteins and cations. (cf. BOOY and BUNGENBERG DE JONG, 1956). In this system easily interchangeable cations are present, which neutralize the negative electric charges of the system. Exchange of a cation for another cation might alter the electric attractions in the membrane and thus cause an alteration in the permeability. (BOOY 1956).

Through the modelexperiments we can get only a few indications about some elements from which the membrane is built up. DE HAAN (1935) found some indications in favour of the complex theory. As a matter of fact he found that small concentrations $Ca(NO_3)_2$ and $Co(NH_3)Cl_3$ have a condensing influence on the plasm. Small concentrations of a trivalent cation with a monovalent anion would influence the waterpermeability more strongly than a bivalent cation with a monovalent anion. Bivalent cations would have a stronger effect than a monovalent cation. In the following sequence the influence on the waterpermeability decreases $3\text{–}1 > 2\text{–}1 > 1\text{–}1$. This would be in correspondence with the hypothesis that in this order also the compensation of the surplus of negative charges in the phosphatide layers would decrease. This would in its turn result in a more or less strong condensing of the plasm.

From Fig. 22 it appears that also in the experiments with chlorides carried out here a trivalent cation (La) has a stronger effect than the

bivalent cation (Ca) and this in its turn a stronger effect than a monovalent cation.

The above mentioned sequence given for the water permeability therefore also holds good for the influence on the active chloride uptake. According to the theory of BOOY and BUNGENBERG DE JONG one might imagine that the sodium or calcium in the cytoplasm alters the electric charge of the plasm membrane, causing the decrease in permeability for the chloride ions. When we again transfer the leaf-segments to a potassium chloride solution the rate of uptake of the chloride ions increases slowly. To what extent a partial exchange of sodium or calcium ions against potassium ions is the cause of this slow increase in chloride uptake, we do not know. On exchange of the various cations one would expect a quicker increase. The monovalent cations prove to inhibit the chloride uptake as follows: $Li > Na > K$. So the behaviour of the monovalent cations just as that of the ions of a different valence, might be an indication of the fact that the structure of the plasm is modified by a change in charge. On our investigating the bivalent cations Ca, Mg and Sr the following sequence for the inhibitory action was found, $Sr > Ca = Mg$. This might be caused by alterations in structure as discussed above.

So, the results obtained in the experiments with a pretreatment with different salt solutions may be explained by assuming certain alterations in structure in plasm or in plasm membranes, which can be brought about by these salt solutions. There have been found some points of resemblance with BUNGENBERG DE JONG's theory which might indicate that phosphatide systems act an important part in this.

An other possibility for the explanation of the cation effects described above is an influence on the formation of the mitochondria. FLORELL (1956, 1957) found that calcium has a direct influence on the formation of mitochondria in excised barley roots. This would cause an increase in the amount of mitochondria, which might explain the increase in nitrate uptake found by BURSTRÖM (1954). This agrees with ROBERTSON's opinion (1951) that the mitochondria would act an important part in the uptake of anions.

By a pretreatment with calcium chloride the chloride uptake from the potassium chloride solution was diminished in our experiments. If the calcium has an influence on the formation of the mitochondria we should therefore expect according to FLORELL and ROBERTSON an increase in the uptake of anions.

FLORELL also mentions the possibility of calcium having an influence on the permeability of the membranes of the mitochondria. If so calcium decreases the permeability in our experiments, whereas it increases the permeability in FLORELL's experiments.

VIETS (1944) investigated the influence of different polyvalent cations on the absorption of K and Br in excised barley roots. Calcium has a stimulating effect on the uptake of potassium and bromide. From Fig. 34 it appears that in our experiments too the chloride uptake was stimulated by the presence of calciumsulphate. VIETS, however, found that a pretreatment in an aerated calcium sulphate solution for four

hours had no effect. Neither had a pretreatment with $CaSO_4$ any effect in our experiments. According to VIETS the calcium could work primarily on the plasm membrane or on a surface metabolism closely connected with permeability.

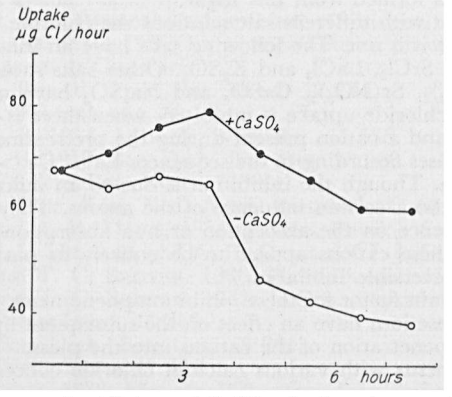


Fig. 34. The influence of addition of $CaSO_4$ during the uptake on the chloride absorption. Uptake from 0,001 M KCl or from 0,001 M KCl + $CaSO_4$.

OVERSTREET and others (1952) found that potassium is more firmly bound when it is absorbed in the presence of calcium. JACOBSON and others (1950), FAWZY, OVERSTREET and others (1954) found that a loss of K, Ca and other cell components induced by a high H-ions concentration, can be partly or entirely inhibited by addition of K or Ca to the acid medium. In our experiments it was found that also Ca influences the loss of chloride ions during the pretreatment in an acid medium. (see Chapter 7).

SUMMARY

The results of the preceding research show that the rate of uptake of chloride ions by *Vallisneria* leaves, may be influenced by various pretreatments of the material during a shorter or longer period.

By the method described in Chapter 2 the chloride uptake per hour was determined.

If during a pretreatment in water the *Vallisneria* leaves are exposed to light, the rate of chloride uptake increased, irrespective of the fact whether the uptake occurs in the light or in the dark. By our aerating with air free from CO_2 during the pretreatment as well as during the uptake the formation of carbohydrates in the light has been inhibited as much as possible. The effect of the light pretreatment is comparatively strongest if subsequent uptake takes place in the dark. If this effect is due to the formation of a specific substance during the pretreatment in the light, it follows from this investigation that it continues to be active for at most 24 hours. Sometimes there is not any effect left after 12 hours. A pretreatment of 3–5 hours in water in the light is sufficient to give an appreciable increase in chloride uptake.

Administration of sugar during the pretreatment or during the uptake influences the course of subsequent uptake. The effect of the specific substance formed under the influence of light during the pretreatment is not changed by this addition of sugar.

Sugar administered during the pretreatment may influence the subsequent chloride uptake in two ways; 1) by an inhibitory action during the first few hours of the uptake period, 2) by a stimulating influence after this initial period. The

first effect may be due to a dehydration of the plasm, which alters the conductivity for ion transport. The second effect may be a metabolic influence. The absorbed sugar might supply energy via metabolism for the accumulation of chloride ions.

Also carbohydrates formed in photosynthesis can stimulate the chloride uptake. This appears to depend on the amount of sugar already present in the material and on the products formed from this sugar.

By a pretreatment with different salt solutions the chloride uptake is sometimes inhibited, sometimes it is not. The following salts have an inhibitory effect: NaCl, LiCl, $CaCl_2$, $MgCl_2$, $SrCl_2$, $LaCl_3$ and K_2SO_4. Other salts such as KNO_3, $NaNO_3$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, $CaSO_4$ and Na_2SO_4 have no effect. From this it appears that the chloride uptake is inhibited, when there is a particular combination of an anion and a cation present during the pretreatment. The strength of the inhibition decreases according to the sequences La > Ca > Na, Li > Na > K and Sr > Mg = Ca. Though the inhibition is due to an influence of the cations, yet there must also be a certain influence of the anions. They may be significant through theire influence on the absorption or non absorption of the cations. The inhibitions due to these cations appear to be entirely or partly reversible. Only $LaCl_3$ causes an irreversible inhibition.

Light is an important factor for these inhibition phenomena. Only by a pretreatment in the light these ions have an effect on the subsequent uptake. Here too this may be due to the penetration of the cations into the plasm.

From the experiments with various calcium chloride concentrations it appears that the inhibition grows stronger according as the concentration increases.

Also the duration of this $CaCl_2$ pretreatment is significant. This indicates an influence of the amount of ions absorbed in the plasm.

The pH during the pretreatment proves to influence the subsequent chloride uptake. Only at pH 3 chloride is released during the pretreatment. Calcium inhibits this release but it has no influence on the effects of various pH's on subsequent chloride absorption.

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