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INFLUENCE OF LIGHT AND SUCROSE ON THE UPTAKE AND TRANSPORT OF CHLORIDE IN VALLISNERIA LEAVES

BY

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INTRODUCTION AND METHOD

The problem discussed in this publication is the influence of light and sucrose on uptake and transport of chloride ions. The system used for the experiments has been kept as simple as possible.

In order to study the uptake of chloride ions parts of leaves 2.5 cm long and 4 mm wide have been used in series of 8, absorbing chloride from solution or from agar. The series were kept in the dark or they were exposed to light, sugar or some other substance being added to the medium or not. The solutions were constantly being aerated with air free from carbon dioxide. The temperature amounted to 25° C. For examining the transport 5 cm leaflengths have been used in boxes of opaque black perspex, which were divided into two parts by a partition slotted for the leaflengths. The first zone of 2.5 cm of the leaflengths which is called the absorbing part in this paper is placed between agar strips or immersed in a solution containing KCl with $CaSO_4$ together with substances as sugars, mannitol and inositol, the influence of which on uptake and transport can be examined. The second 2.5 cm of the leaflengths, the free part, is placed in the adjoining compartment of the perspex box between agar plates or in a solution, if required with addition of other substances. The uptake from agar and the one from solution have always been used side by side for checking purposes. An advantage of the agar method is the local administration, the protection of the leaftissue from drying out, the proper aeration and the effective prevention of the contents of the two compartments from being mixed. In using fluids a passage through the slots in the partition is prevented by filling them up with vaseline mixed with carbon particles. The compartments can be covered with transparent or black perspex plates.

In experiments with closed perspex boxes the quantity of carbon dioxide present is rather small. Respiration produces also some carbon dioxide which can be used in photosynthesis. The quantity of carbohydrates synthesized in the boxes will be rather small especially if the solutions used are free from carbon dioxide and aerated with carbon dioxide free air; on the other hand it cannot be proved that they have had no influence at all on the accumulation processes.

For the last few years *Vallisneria* has been cultivated in a cellar with exposure to artificial light in concrete tanks filled with water purified by ion exchangers. The leaves poor in chloride can take up great quantities of chloride in the light. The agar used was Difco agar, specially purified for the purpose.[1])

It is our experience that agar raises the uptake of chloride somewhat. This may be attributed to carbo-hydrates being present. The preparation of the leaf material entirely corresponds with that of previous investigations. Before the experiments the leaves were well washed and from their sides so much was cut off that the width everywhere amounted to 4 mms. Next they were cut with a sharp knife into smaller pieces of 2.5 or 5 cms and put in distilled water. Eight series are composed from 8 identical leaf pieces taken from 8 different leaves and from different parts of these leaves. The pieces of one series are arranged in a perspex frame so that they don't overlap. Before the uptake begins the leaf segments in their frame remain in aerated distilled water for 24 hours in order to eliminate the wound effect caused by the cutting.

I. *Influence of light and sucrose on the uptake in 2.5 cm leaf segments*

The uptake of chloride in the dark varies a great deal in strength. Per series it may amount from 0 to more than 200 μg which depends on the pretreatment, particularly the exposure and the supply of carbohydrates. By addition of sucrose to the medium the uptake may be increased considerably (Table 1). The material of these experiments

TABLE 1

Influence of sucrose and light on the uptake of chloride. Pretreatment 24 hours' exposure to light. Uptake from 1/1000 M KCl + $CaSO_4$ solution. The molarity relates to the KCl. In exp. 835 uptake from agar with 1/500 M KCl + $CaSO_4$. Instead of sucrose 1/20 M 2 % fructose has been added to the agar. In all tables uptake is given in μg Cl per 8 leaf segments of 2.5 cm length and 0.4 cm width per 24 hours at 25°C.

	Exp. 1004	Exp. 1006	Exp. 1007	Exp. 1086	Exp. 835
dark	99	78	110	153	36
dark + sucr. 1/20 M	256	220	195	185	43
light — CO_2	568	401	461	469	193
light + sucr. 1/20 M	618	504	561	540	267

showed a rather strong uptake in the dark of $\pm$ 100 μg. By administration of sucrose 1/10 M it rises to 256 μg.

The material was pretreated at 100 f.c. white light for 24 hours. Exposure during the uptake to 100 f.c. has a stronger effect than administration of sucrose. During the exposure air free from carbon dioxide was led through the solution. This causes an increase of pH and a disappearance of CO_2 and bicarbonate from the solution. It

[1]) We acknowledge our indebtedness to the Difco Laboratories Detroit (Mich). for their help in providing the purified agar and to the Wester Suikerraffinaderij for placing purified sucrose at our disposal.

may be presumed that in this way the formation of carbo-hydrates in the photosynthesis is for the most part inhibited (ARISZ 1947).

If besides exposure sucrose is added, the uptake becomes greater yet. Fructose has an identical influence as sucrose (Table 1). Also mannitol and inositol stimulate the uptake of chloride in light and dark (Table 2).

From Table 3 it appears that addition of sucrose to about 0.24 M

TABLE 2

Influence of inositol on the chloride uptake from 1/1000 M KCl + $CaSO_4$

	In the light	In the dark
24 hours uptake	504	43
„ „ with 0.04 M inositol	513	43
„ „ „ 0.06 „ „	563	46
„ „ „ 0.09 „ „	589	60
„ „ „ 0.15 „ „	660	71

TABLE 3

Influence of sucrose on the uptake of chloride in the light. Pretreatment 24 hours in the light. Uptake 24 hours from 1/1000 M KCl + $CaSO_4$. (Exp. 900)

24 hours uptake from 1/1000 M KCl + $CaSO_4$	245
with addition of 0.02 M sucrose	308
0.04 „ „	318
0.08 „ „	353
0.16 „ „	370
0.24 „ „	391
0.32 „ „	*184*

results in an increase of the uptake of chloride ions in the light. At a still higher sugar concentration, however, the uptake decreases, because at $\pm$ 0.3 M sucrose plasmolysis takes place. As the protoplasts are separated by plasmolysis, it is comprehensible that the uptake into and the transport to the cells not adjoining the medium greatly decrease. In that case a symplasmatic transport is of course no more possible. This sets a limit to the concentration of sucrose, from which a favourable effect on the uptake may be expected.

It may be that the sugars glucose, fructose and sucrose behave differently. They are taken up into the cells, where they are converted. One sugar is transported better than another. The differences, however, have not been fully investigated.

To discover whether the influences of light and sucrose are of the same nature the following experiment has been made. In it we started from the notion, that if the influence of sugar and of light are identical, a further addition of sucrose will not have any influence on the salt uptake with light saturation. For this purpose *Vallisneria* material has been pretreated in the dark for 24 hours and next exposed to 150, 200 and 300 f.c. white light for 24 hours chloride uptake. (Table 4 experiment 1089). It appears that already at 150 f.c. light saturation has set in. Addition of 1/10 M sucrose, however, results as well at 150, at 200 as at 300 f.c. in a further uptake of more than 100 μg Cl.

This indicates that the influence of exposure and addition of sugar cannot be based on the primary formation of one and the same substance (cf. p. 243).

TABLE 4

Influence of exposure to light and sucrose addition on the uptake of chloride. Pretreatment 5 hours in the dark. Uptake from a 1/1000 M KCl + $CaSO_4$ solution during 24 hours; aerated with carbondioxide free air. (Exp. 1089)

dark	186
dark + sucrose	232
exposure 150 f.c.	264
„ 150 f.c. + sucrose	384
„ 200 f.c.	267
„ 200 f.c. + sucrose	370
„ 300 f.c.	257
„ 300 f.c. + sucrose	367

II. *Influence of exposure to light of the absorbing zone in 5 cm leaf lengths*

In Table 5 data have been gathered on the influence of exposure of the absorbing zone on the accumulation of chloride ions in the absorbing and in the free leaf part. Two cases are to be distinguished: the free zone of the leaf may be exposed to light or kept in the dark.

Only a few experiments will be discussed more fully. Experiment 1087 is remarkable. In series 3 the absorbing and the free part are both in the dark, sucrose being added to both. On comparison with series 1 of the same experiment this addition of sucrose to the free part in the dark appears to have resulted in a slight promotion of the uptake. If the absorbing part is exposed (series 4) the accumulation in the absorbing part increases from 160 to 408 μg, that in the free part from 63 to 178 μg. So the total uptake increases from 223 to 586 μg. It is interesting that in this case the free part also accumulates more and that only on exposure of the absorbing part the stimulating action of the sucrose in the free part has a stronger effect.

From this it appears that exposure of the absorbing part increases the supply of ions to the symplasm and makes a stronger accumulation possible both in the absorbing and in the free part.

In experiment 1014 series 1 and 2 exposure of the absorbing part increases the accumulation in the free part in the dark from 82 to 146 μg, while in the absorbing part itself the accumulation increases from 117 to 373 μg.

Exposure of the absorbing zone, the free zone being in the light (experiment 1014 series 3 and 4) likewise results in an increase of accumulation in both zones; in the absorbing zone there is an increase of 142 to 475 μg, in the free zone from 46 to 280 μg. Here therefore the accumulation in the free part is restricted by the slight uptake in the absorbing zone which is in the dark. This indicates that exposure to light raises the chloride ion concentration in the symplasm and that as a result the accumulation in the free part in the dark, in the light and in the dark with sucrose increases. Experiment 1026 gives the same picture. Experiments 1024 and 1025 show a bad transport,

TABLE 5

Influence of exposing the absorbing part to light. Pretreatment with 1/20 M fructose in the light (1025 and 1026), with sucrose (1024), others water. Uptake from agar with 1/100 M KCl + $CaSO_4$, S 41 from 0.002 M KCl solution. Addition of 1/20 M sucrose; 24 hours.

series	exposure	Exp. 1014	Exp. 1024	Exp. 1025	Exp. 1026
1	dark — dark	117 — 82 (199)	103 — – 3 (100)	110 — – 3 (107)	146 — 56 (202)
2	*light* — dark	**373 — 146** (519)	**199** — – 7 (192)	**200** — **66** (266)	**454** — 46 (500)
3	dark — light	142 — 46 (188)	121 — – 7 (114)	102 — 67 (169)	156 — 78 (234)
4	*light* — light	**475 — 280** (755)	**197 — 35** (232)	**334 — 110** (444)	**589 — 295** (884)

		Exp. 1087		Exp. 1088
1	dark + sucr. — dark	127 — 53 (180)	dark + sucr. — light	250 — 44 (294)
2	*light* + sucr. — dark	**284 — 100** (384)	*light* + sucr. — light	**350 — 55** (405)
3	dark + sucr. — dark + sucr.	160 — 63 (223)	dark + sucr. — light + sucr.	263 — 51 (314)
4	*light* + sucr. — dark + sucr.	**408 — 178** (586)	*light* + sucr. — light + sucr.	**392 — 69** (461)
5	dark — dark + sucr.	120 — 63 (183)	dark — light + sucr.	239 — 51 (290)
6	*light* — dark + sucr.	**252 — 114** (366)	*light* — light + sucr.	**321 — 44** (365)

TABLE 5A

Localising effect of sucrose

	Exp. S 41
dark — light	169 — 89 (258)
light — light	**344 — 181** (525)
dark + sucr. — light	238 — 96 (334)
light + sucr. — light	**451** — 96 (547)

as has repeatedly been found in *Vallisneria* leaves. Yet the effect of exposure of the absorbing part is noticeable here too in a stronger accumulation in the free part. In experiment 1088 transport is also slight in all circumstances. Here again it is a different factor, the conductivity of the symplasm that keeps the transport on a low level and as a result limits the accumulation in the free part.

From these experiments it follows that on uptake in the dark the accumulation in the free zone is limited by the supply of chlorides in the symplasm of the absorbing part.

Exposure of the absorbing part increases the supply of ions to the symplasm of the absorbing part and by doing so it raises the accumulation both in the absorbing and in the free part.

Just as the experiments with inhibitors mentioned in a previous publication (ARISZ 1953, 1956) and the experiments on redistribution (1954, 1956) these experiments are a proof of the fact that in Vallisneria leaves the transport of chloride ions to the free part of the leaf takes place in a symplasm and not in the cell walls. For in that case an exposure to light of the absorbing zone could never have such a decisive influence on the accumulation in the free part.

III. *Influence of exposure to light of the free zone*

III A. Two cases are to be distinguished. In the first the absorbing part is in the light (Table 6). In this case exposure of the free part as a rule causes an increase in accumulation in this zone, which may sometimes be considerable (16 times an increase has been found, once a slight decrease). In the presence of sucrose the accumulation in the dark is mostly fairly great in the free zone, so that the influence of exposure grows less distinct. It is, however, remarkable, that exposure of the free part sometimes causes a considerable increase up to 50 % or more of the accumulation in the absorbing part (Exp. 1010 series 3 and 4, 5 and 6, exp. 1083 series 7 and 8, exp. 1014, 1025 and 1026). In experiment 1112 the free part of the leaf was in distilled water. During exposure to light it was aerated with carbon dioxide free air. This means that during exposure formation of carbo-hydrates was for the greater part prevented. *This is an important observation which shows that from the exposed free part there proceeds an influence on the absorbing zone, which increases the accumulation there in spite of the absorbing part already being in the light.* So exposure to light of the free zone has two results: a stronger accumulation on the spot and a stronger accumulation in the absorbing zone (Table 21).

III B. In the second case the absorbing part is left in the dark. (Table 7). Here again exposure of the free zone results as a rule in an increase of the accumulation in the absorbing and in the free zone provided sucrose has been administered to the absorbing and to the free zone at the same time. If, however, the absorbing zone is in the dark and does not get any sucrose the effect is variable (Table 7). As a rule the accumulation in the absorbing part increases, while the chloride ion concentration in the free part also increases, but in

TABLE 6

Influence of exposure to light of the free part, the absorbing part being in the light. Uptake from agar with 1/100 M KCl + $CaSO_4$; addition of 1/20 M sucrose, 24 hours.

	Exp. 1010	Exp. 1083	Exp. 1013	Exp. 1112
light — dark	351 — 138 (489)	209 — 50 (259)	366 — 89 (455)	160 — 75 (235)
light — *light*	**376 — 185** (561)	**263 — 131** (394)	**401 — 209** (610)	**196 — 92** (288)
light — dark + sucr.	355 — 135 (490)	273 — 117 (390)		189 — 116 (303)
light — *light* + sucr.	**508 — 238** (746)	**316 — 135** (451)		196 — **153** (349)
light + sucr. — dark	413 — 138 (551)	228 — 107 (335)		227 — 96 (323)
light + sucr. — *light*	**497 — 160** (657)	235 — **135** (370)		**252 — 167** (419)
light + sucr. — dark + sucr.	462 — 281 (743)	238 — 110 (348)		284 — 128 (412)
light + sucr. — *light* + sucr.	**511** — 241 (752)	**341 — 128** (469)		**352 — 210** (562)
	Exp. 1024	Exp. 1025	Exp. 1026	Exp. 1014
light — dark	199 — – 7 (192)	200 — 66 (266)	454 — 46 (500)	373 — 146 (519)
light — *light*	197 — **35** (232)	**334 — 110** (444)	**589 — 295** (884)	**475 — 280** (755)

TABLE 7

Influence of exposure to light of the free part, the absorbing part being in the dark. Uptake from 1/1000 M KCl + $CaSO_4$ solution (1004, 1006 and 1007), S 18, S 19 and S 21 from 0.002 M solution; from agar with 1/100 M KCl + $CaSO_4$ (1014, 1024, 1025, 1026).

	Exp. 1004	Exp. 1006	Exp. 1007	Exp. 873	S 21 L	Exp. 1094
dark — dark	121 — 125 (246)	128 — 64 (192)	71 — 14 (85)		64 — 7 (71)	82 — 18 (100)
dark — *light*	**135** — *7 (142)*	*85* — *43 (128)*	**85** — **35** (120)		**103** — 10 (113)	**153** — **75** (**228**)
dark + sucr. — dark	156 — 50 (206)	206 — 50 (256)	199 — 64 (263)	49 — 3 (52)		139 — 25 (164)
dark + sucr. — *light*	**224** — **71** (295)	**263** — 57 (**320**)	**220** — **85** (**305**)	**158** — **28** (**186**)		**202** — **110** (**312**)
	Exp. 1024	**Exp. 1025**	**Exp. 1026**	**Exp. 1014**	**S 18H**	**S 19H**
dark — dark	103 — −3 (100)	110 — −3 (107)	146 — 56 (202)	117 — 82 (199)	67 — 3 (70)	99 — 14 (113)
dark — *light*	**121** — −7 (114)	102 — **67** (**169**)	**156** — **78** (**234**)	**142** — *46 (188)*	63 — **56** (**119**)	**116** — **99** (**215**)

TABLE 7A

	Exp. 1118
dark — dark	139 — 50 (189)
dark — *light*	**178** — **86** (264)
dark + sucr. — dark	150 — 57 (207)
dark + sucr. — *light*	**228** — **103** (331)
dark — dark + sucr.	143 — 72 (215)
dark — *light* + sucr.	**188** — **114** (302)
dark + sucr. — dark + sucr.	153 — 86 (239)
dark + sucr. — *light* + sucr.	**249** — **121** (370)

exp. 1004 series 2 and 1006 series 2 it decreases. This phenomenon has only been stated a few times.

In Table 7 A (exp. 1118) the exposed free part was in distilled water and aerated with carbon dioxide free air. In this experiment the influence of the formation of carbo-hydrates in the light was for the greater part prevented. The increased accumulation in the absorbing zone is the result of a specific substance formed in the exposed free part and transported to the absorbing zone.

In previous experiments (1947, 1948 and 1953) on material cultivated in the day light, we repeatedly found that the accumulation in the exposed free zone was greater than in the absorbing part that was kept in the dark. As an example we give Table 8 (cf. ARISZ 1947 Table 7 and 1953 figs. 8A and 11A). This may be explained by assuming that

TABLE 8

Influence of exposing the free part of the leaf to light.

	Exp. 832
A. Uptake from 1/500 M KCl + $CaSO_4$ solution in the light	75
Uptake from 1/500 M KCl + $CaSO_4$ solution in the light + 2 % fructose	150
Absorbing part in the dark + fructose, free part in the light + fructose	29 — 133 (162)
	Exp. 821
B. Absorption by a 2.5 cm leaf segment in the dark + 2 % fructose	30
Absorption by a 2.5 cm leaf segment in the light + 2 % fructose	128
5 cm leaf segment; absorbing part in the dark + 2 % fructose / free part in the dark + 2 % fructose	17 — 7 (24)
5 cm leaf segment; absorbing part in the dark + 2 % fructose / free part in the light + 2 % fructose	65 — 57 (122)

in this case transport is fast in the symplasm so that with the restricted uptake in the dark, the accumulation in the absorbing part is held down in favour of that in the free part. Scarcity of carbo-hydrates in the absorbing zone may promote this. In Table 8B the uptake in the dark of a 2.5 cm leafsegment in the presence of fructose is 30 μg, through exposure it increases to 128 μg. If the absorbing and the free zone of a 5 cm leaflength are both in the dark, fructose being present in the medium, there is hardly any accumulation in the free zone (7 μg). If, however, the free part of a 5 cm leaflength is exposed to light, we find a total uptake of 122 μg, apportioned between 65 μg in the absorbing and 57 μg in the free zone. In this case exposure of the free zone caused a rise in the total increase from 24 to 122 μg. This is fairly equal to the uptake of a 2.5 cm leaflength exposed to light. Here therefore the effect of an exposure of the free zone on the total uptake is equally great as the effect of an exposure on the uptake of

a segment of 2.5 cm length. This also shows an influence of exposure of the free zone on the uptake from the medium.

IV. *Influence of addition of sucrose to the absorbing part in the light*

IV A. We shall now discuss experiments in which the influence of *sucrose* has been investigated. This may be administered to the absorbing or to the free zone, while moreover the prevailing exposure may have an influence on the effect.

Table 9 contains data in the case the free zone is in the light or in the dark, sucrose being present or not. The result is clear. There always occurs an increase of accumulation in the absorbing zone; there is an increase in the free part in 10 of the 17 exp., but in other cases the latter continues unaltered. In exp. 1010 and S 41 there is a decrease. In most cases the total uptake increases considerably.

IV B. Remarkable is the influence of adding sucrose to the absorbing part, while it is in the dark (Table 10). The total uptake of Cl increases, but less so than if the absorbing zone is exposed to light. The accumulation in the absorbing part always increases. The results concerning the accumulation in the free part, however, vary in this case. In the 26 experiments eleven times a decrease, eight times an increase was found; in the other cases there was no change. This tendency to a decrease of accumulation in the free part must be due to the increased competition exercised by the plasm adjoining the vacuoles in the absorbing part. It accumulates chloride ions from the symplasm into the vacuoles, owing to which fewer chloride ions remain available for transport to the free part. Though by the sucrose the total uptake of chloride ions from the medium is increased there is a competition between the accumulation in the absorbing and that in the free part, since the uptake is restricted in the dark.

V. *Influence of addition of sucrose to the free part*

Table 11 gives the results of administering sucrose to the free part, the absorbing zone being exposed to the light. The promoting influence of sucrose is as a rule small and local, but in some cases there is an increased accumulation in the absorbing part too. In experiment 1083 the accumulation in the absorbing zone decreases in 2 cases, in two others it increases. The differences are not great in this experiment. The total uptake remains equal in many cases, but it may sometimes increase.

Table 12 contains the results of 23 observations on the influence of administering sucrose to the free leaf zone, the absorbing zone being in the dark. It is a striking fact that in eleven cases the total uptake (accumulation in absorbing and free parts together) decreases strongly whereas in three it remains fairly equal and in nine increases slightly. Especially if the absorbing zone is in the dark without a supply of sucrose, the phenomenon occurs that on sucrose addition to the free part, the chloride accumulation in the absorbing part diminishes in

TABLE 9

Influence of sucrose addition to the absorbing part, the free part being in the light. Uptake from agar with 0,01 M KCl + $CaSO_4$, S 41 from a 0.002 M solution; addition of 0.05 M sucrose, 24 hours.

	Exp. 1013	Exp. 1010	Exp. 1083	S 41	Exp. 1112
light — light	401 — 209 (610)	376 — 185 (561)	263 — 131 (394)	344 — 181 (525)	196 — 92 (288)
light + *sucr.* — light	**586** — **366** (952)	**497** — *160* (657)	**316** — 135 (451)	**451** — *96* (547)	**252** — **167** (419)
				Exp. 1088	
light — light + sucr..	401 — 259 (660)	508 — 238 (746)	235 — 135 (370)	321 — 44 (365)	196 — 153 (349)
light + *sucr.* — light + sucr..	**586** — **486** (1072)	511 — 241 (752)	**341** — 128 (469)	**392** — **69** (461)	**352** — **210** (562)
light — dark		351 — 138 (489)	209 — 50 (259)		160 — 75 (235)
light + *sucr.* — dark		**413** — 138 (551)	**273** — **117** (390)	Exp. 1087	**227** — **96** (323)
light — dark + sucr..		355 — 135 (490)	228 — 107 (335)	252 — 114 (366)	189 — 116 (305)
light + *sucr.* — dark + sucr..		**462** — **281** (743)	238 — 110 (348)	**408** — **178** (586)	**284** — **128** (412)

TABLE 10

Influence of sucrose addition to the absorbing part, the free part being in the dark. Uptake from agar with 0.01 M KCl + $CaSO_4$ (1087, 1088), 0.002 M KCl + $CaSO_4$ (873, 874, 878, 879), from 0.001 M KCl + $CaSO_4$ solution (1004, 1006, 1007), from 0.002 M solution S 8 and S 9; 24 hours.

	Exp. 873	Exp. 874	Exp. 878	Exp. 879	S 9H	S 8L
dark — light	98 — 46 (144)	88 — 60 (148)	80 — 87 (167)	53 — 84 (137)	57 — 50 (107)	131 — 17 (148)
dark + *sucr.* — light	**221** — *21* (242)	**197** — *46* (243)	**186** — *45* (231)	**76** — *53* (129)	**85** — *14* (99)	**258** — *11* (269)
dark — light + sucr..	53 — 67 (120)	35 — 53 (88)	102 — 68 (170)	30 — 37 (67)	7 — 64 (71)	116 — 17 (133)
dark + *sucr.* — light + sucr..	**158** — *28* (186)	**151** — *21* (172)	**186** — **102** (288)	**91** — **64** (155)	**75** — *50* (125)	**180** — *– 3* (177)
	Exp. 1087					Exp. 1118
dark — dark + sucr..	120 — 63 (183)					143 — 72 (215)
dark + *sucr.* — dark + sucr..	**160** — 63 (223)					**153** — **86** (239)
		Exp. 1004	Exp. 1006	Exp. 1007	Exp. 1094	
dark — light		135 — 7 (142)	85 — 43 (128)	85 — 35 (120)	153 — 75 (228)	178 — 86 (264)
dark + *sucr.* — light		**224** — **71** (295)	**263** — **57** (320)	**220** — **85** (305)	**202** — **110** (312)	**228** — **103** (331)
	Exp. 1088					
dark — light + sucr..	239 — 51 (290)	7 — 106 (113)	156 — 99 (255)	7 — 104 (111)		188 — 114 (302)
dark + *sucr.* — light + sucr..	**263** — 51 (314)	**248** — 99 (347)	**241** — 99 (340)	**213** — *85* (298)		**249** — 121 (370)
dark — dark					82 — 18 (100)	139 — 50 (189)
dark + sucr. — dark					**139** — 25 (164)	**150** — 57 (207)

TABLE 11

Influence of sugar addition to the free part, the absorbing part being in the light. Uptake from agar with 0.01 M KCl + $CaSO_4$ 24 hours, sucrose 0.05 M.

	Exp. 1013	Exp. 1010	Exp. 1083	Exp. 1088	Exp. 1112
light — light	401 — 209 (610)	376 — 185 (561)	263 — 131 (394)		196 — 92 (288)
light — light + *sucr.*	401 — **259** (660)	**508** — **238** (746)	*235* — 135 (370)		196 — **153** (349)
light — dark		351 — 138 (489)	209 — 50 (259)		160 — 75 (235)
light — dark + *sucr.*		355 — 135 (490)	**228** — **107** (335)		**189** — **116** (305)
light + sucr. — light	586 — 366 (952)	497 — 160 (657)	316 — 135 (451)	350 — 55 (405)	252 — 167 (419)
light + sucr. — light + *sucr.*.	586 — **486** (1072)	511 — **241** (752)	**341** — 128 (469)	**392** — **69** (461)	**352** — **210** (562)
				Exp. 1087	
light + sucr. — dark		413 — 138 (551)	273 — 117 (390)	284 — 100 (384)	227 — 96 (323)
light + sucr. — dark + *sucr.*.		**462** — **281** (743)	*238* — 110 (348)	**408** — **178** (586)	**284** — **128** (412)

TABLE 12

Influence of sugar addition to the free part, the absorbing part being in the dark. Uptake from agar with 0.002 M KCl + $CaSO_4$ (873, 874, 878, 879), from agar with 0.01 M KCl + $CaSO_4$ (1087, 1088), from 0.001 M KCl + $CaSO_4$ solution (1004, 1006, 1007); S 7, S 8, S 9 from 0.002 M solution.

	Exp. 873	Exp. 874	Exp. 878	Exp. 879	Exp. 1004	Exp. 1006	Exp. 1007
dark — light	98 — 46 (144)	88 — 60 (148)	80 — 87 (167)	53 — 84 (137)	135 — 7 (142)	85 — 42 (127)	85 — 35 (120)
dark — light + *sucr.*.	*53* — **67** (*120*)	*35* — 53 (*88*)	**102** — *68* (170)	*30* — *37* (*67*)	*7* — **105** (*113*)	**156** — **99** (**255**)	*7* — **104** (111)
dark + sucr. — light	221 — 21 (242)	197 — 46 (243)	186 — 45 (231)	76 — 53 (129)	224 — 71 (295)	263 — 57 (320)	220 — 85 (305)
dark + sucr. — light + *sucr.*.	*158* — 28 (*186*)	*151* — *21* (*172*)	186 — **102** (**288**)	**91** — 64 (**155**)	**248** — **99** (**347**)	*241* — **99** (**340**)	213 — 85 (298)
	Exp. 901	S 8L	S 7H	S 9H			
dark — light	126 — 36 (162)	131 — 17 (148)	103 — 111 (214)	57 — 50 (107)			
dark — light + *sucr.*.	*66* — **57** (*123*)	*116* — 17 (*133*)	*11* — *60* (*71*)	*7* — **64** (*71*)			
	Exp. 1088						
dark + sucr. — light	250 — 44 (294)	258 — 11 (269)	46 — 4 (50)	85 — 14 (99)			
dark + sucr. — light + *sucr.*.	**263** — 51 (**314**)	*180* — *3* (*183*)	*4* — **89** (**93**)	*75* — **50** (**125**)			
	Exp. 1087						
dark + sucr. — dark	127 — 53 (180)						
dark + sucr. — dark + *sucr.*.	**160** — **63** (**223**)						

9 of the 11 cases. The accumulation in the free part is rather variable, it either increases or decreases.

If sucrose has been administered to the absorbing part a decrease of the accumulation in that zone occurs in 6 cases out of 12, in four cases there is an increase, in two no change. The total quantity accumulated in the two zones together increases in 8 and decreases only in 3 cases. This indicates that administration of sucrose to the absorbing part diminishes the long distance effect of the sugar addition to the free part.

This phenomenon of a long distance effect of sucrose addition to the free part on the accumulation in the absorbing part was quite unexpected and has therefore been carefully investigated.

Mrs. Knobbe and Miss Schreuder made observations about this phenomenon in the years 1953 and 1955. In order to eliminate the influence of the person who makes the determinations, the experiments have been repeated by the junior author (exp. marked S). The phenomenon appeared to have a good reproducibility. It is not so variable as some other reactions of *Vallisneria*, which are greatly dependent on the nutritional condition. It has appeared (ARISZ 1955; ARISZ and SCHREUDER 1956) that this phenomenon has an *osmotic cause*. Table 13 contains two experiments pointing in this direction,

TABLE 13

Influence of sucrose concentration added to the free part, the absorbing part being in the light. Uptake from agar with 0.002 M KCl + $CaSO_4$ (899) from 0.002 M KCl + $CaSO_4$ solution (S 15).

	Exp. S 15L	Exp. 899
dark — light	**103** — 3 (106)	72 — 38 (110)
dark — light + 0.005 M sucr.	**117** — 39 (156)	
dark — light + 0.020 M sucr.	*88* — 17 (105)	
dark — light + 0.040 M sucr.		*45* — 24 (69)
dark — light + 0.060 M sucr.	*74* — 17 (91)	*31* — 17 (48)
dark — light + 0.090 M sucr.		*31* — 21 (52)
dark — light + 0.100 M sucr.	*46* — 3 (49)	
dark — light + 0.150 M sucr.		*26* — 45 (71)

in which the influence of the concentration of the sucrose solution has been examined. It appears that with increasing sucrose concentration administered to the free part, the accumulation in the absorbing zone decreases.

In Table 14 sucrose in an increasing concentration has been added to the absorbing part, the same sucrose concentration 0.1 M being administered to the free part in all experiments. It appears that according as the sucrose concentration administered to the absorbing part increases the uptake increases too. At 0.1 M added both to the absorbing and to the free part of the leaf the accumulation in the absorbing and in the free zone is again normal and even a little higher than without sucrose being administered. Then sucrose again promotes accumulation.

It may be understood that if to the absorbing part itself sucrose is administered or if it has been placed in the light, the accumulation of chloride ions in the absorbing zone is promoted and the osmotic value of these cells increases. In that case the osmotic action of the

TABLE 14

Influence of increasing the sucrose conc. administered to the absorbing part on the osmotic effect of a sucrose solution in contact with the free part. Uptake from 0.002 M KCl + $CaSO_4$ solution

	Exp. S 23H	Exp. S 45
dark — light	**88** — 3 (91)	
dark — light + 0.1 M sucrose	*17* — 24 (41)	
dark + *0.02* M sucr. — light + 0.1 M sucrose	38 — 52 (90)	
dark + *0.06* M sucr. — light + 0.1 M sucrose	52 — 45 (97)	
dark + *0.10* M sucr. — light + 0.1 M sucrose	**95** — 52 (147)	
dark — light		96 — 32 (128)
dark — light + 0.05 M sucrose		*53* — **53** (106)
dark + *0.02* M sucr. — light + 0.05 M sucrose		**117** — **67** (184)
dark + *0.05* M sucr. — light + 0.05 M sucrose		**167** — 67 (234)
dark + *0.10* M sucr. — light + 0.05 M sucrose		**202** — **74** (276)

sucrose in the free part cannot present itself. Sucrose administered to an absorbing leaf zone has not, as we have seen, an inhibitory, but a promoting effect on the uptake of chloride ions, as long as plasmolysis does not take place (Table 3).

VI. *Influence of exposure and addition of sugar during pretreatment on the uptake of chloride ions*

The influence of a pre-exposure was already investigated before (ARISZ 1947, p. 1028). It was then demonstrated that exposure during pretreatment increases the next following uptake of chloride. Aeration during the pretreatment with carbon dioxide free air and the withdrawal of carbon dioxide formed by the tissue does not prevent this favourable influence on the following uptake. So this effect has nothing to do with photo-synthesis of carbo-hydrates.

The intensity of the pre-exposure has a great influence.

In the experiments on influence of sucrose on uptake and transport the influence of sucrose on the strength of the chloride uptake has appeared. This gave rise to our bringing the influence of sucrose during pretreatment into our investigation.

Table 15 gives the result of three experiments which have been made on the influence of sucrose and light during pretreatment. The pretreatment took place in the dark, in the dark with addition of 0.1 M sucrose, in light of 150 f.c. in the absence of CO_2 and in light of this intensity with addition of 0.1 M sucrose. In experiment 1091 the uptake took place in two different light intensities, 50 and 150 f.c. in the absence of CO_2. In experiments 1100 and 1102 the uptake was determined in the dark and at 50 f.c. in the absence of CO_2. All determinations have been made in duplicate.

The uptake in the light at 50 and 150 f.c. is increased during the pretreatment by addition of sucrose. Pre-exposure without sucrose in a medium free from CO_2 causes a stronger increase in uptake than addition of sucrose and this light influence is dependent on the intensity

TABLE 15

Influence of exposure to light and of sucrose during the pretreatment on the uptake of chloride by Vallisneria leaves. In exp. 1091 exposure during uptake 50 and 150 f.c. in CO_2 free medium. In exp. 1100 and 1102 uptake in the dark with exposure to 50 f.c. in CO_2 free medium.

pretreatment 20 hours	uptake μg Cl 50 f.c. — CO_2	uptake μg Cl 150 f.c.—CO_2	uptake μg Cl dark		uptake μg Cl 50 f.c. — CO_2	
	Exp. 1091	Exp. 1091	Exp. 1100	1102	Exp. 1100	1102
dark	174	280	71	75	181	362
dark+0.1 M sucrose	309	366	117	117	359	451
light 150 f.c. — CO_2	401	444	220	138	380	540
light+0.1 M sucrose	444	525				

of the light during the pretreatment. In experiment 1091 addition of sucrose during pre-exposure has not a great, but yet distinctly favourable effect. From experiments 1100 and 1102 it follows that the influence of the pretreatment with sucrose and light is likewise noticeable if the uptake of chloride takes place in the dark. This is an important observation, to which we will revert (cf. fig. 3 curves A, B and C).

At the same time it appears from this experiment, which fact has been corroborated by further experiments, that a substance formed in the light can be distinguished from a substance formed in the dark in the presence of sucrose. It is clear that the sucrose is absorbed by the leaf during pretreatment, but what conversion it undergoes in the leaf is unknown. So we distinguish a preformed 'light substance' and a preformed 'sucrose substance'.

It was interesting to know whether the substance formed in the light is transported in the leaf. For this purpose experiments have been made (Table 16), in which one half of the 5 cm leaflengths in perspex boxes was exposed during pretreatment with 150 f.c. in the absence of CO_2, the other half remaining in the dark. It is of course impossible to ascertain that the exposed part did not have any CO_2 at its disposal. Communication of the two compartments was avoided as much as possible, but cannot be quite excluded, the filling up being done with vaseline and carbon particles. Besides there are big intercellular canals with septa in the leaf, so that respiration CO_2 in an exceedingly low concentration may have had some influence. The quantity of sugar, however, which might be formed in this way by photosynthesis is so small and the possibility of its being transported in the leaf to the adjoining part that remains in the dark, so slight, that this possibility may be left out of consideration. In experiment 1099 the exposed compartment was moreover aerated with CO_2 free air.

The 5 cm control leaflengths remained entirely in the dark during

pretreatment. After the pretreatment the 5 cm leaflengths were cut into two equal parts and in the next following uptake from 1/1000 M KCl + $CaSO_4$ exposed, one series to 50, a second series to 150 f.c. Three experiments have been made.

TABLE 16

Influence of pretreatment on the uptake of chloride. Transport of the substance formed during pretreatment in the light. Leaflengths of 5 cm pretreated dark-light or dark-dark. Exposure to 150 f.c. in carbondioxide free water. After the pretreatment the leaflengths have been cut in two parts of 2.5 cm. Uptake by the separated leafsegments in 50 and 150 f.c. from a 0.001 M KCl + $CaSO_4$ solution (μg Cl/24 hours, 8 segments of 2.5 cm)

pretreatment 5 cm leaflengths	2.5 cm parts	uptake	exp. 1092	exp. 1093	exp. 1099
$dark_1$ — light	$dark_1$	50 f.c.	277	284	210
		150 f.c.	309	323	249
	light	50 f.c.	408	369	405
		150 f.c.	430	412	462
$dark_2$ — $dark_3$	$dark_2$	50 f.c.	224	209	153
		150 f.c.	217	227	227
	$dark_3$	50 f.c.	209	209	156
		150 f.c.	217	217	231

The leafzone d 1 which was connected with the exposed zone during pre-exposure, had to be compared during the uptake with the leafsegments d 2 and d 3, which remained in the dark during pre-exposure. It appears from all three experiments that both at 50 f.c. and at 150 f.c. d 1 takes up more chloride than the average amount of d 2 and d 3.

In experiment 1092 this amounts for 50 f.c. to 277 against 216 μg, for 150 f.c. 309 against 217 μg. In experiment 1093 it amounts for 50 f.c. to 284 against 209 μg and for 150 f.c. 323 against 222 μg. In experiment 1099 for 50 f.c. 210 against 155 μg and for 150 f.c. 249 against 229 μg. The experiments make the impression that the substance formed in the light during pretreatment is transportable.

A fairly large number of experiments have been made on the influence of pre-exposure on uptake at various light intensities. In these experiments quite different results have been obtained, which may be understood now that it is known that the sugar condition of the leaves before the experiment also affects the uptake. The experiments cannot be very well compared with each other, as they have been carried out with different plant material. We shall discuss only one of these experiments, in which the influence of pretreatment in the dark and of pre-exposures to 70 and 120 f.c. have been compared. A Philips sodium lamp was used provided with a water filter to remove the heat rays. The uptake of 1/1000 M KCl + $CaSO_4$ was determined in the dark at 20, 40, 70, 120, 160 and 240 f.c. On account of the great number of points that had to be determined in one experiment, these observations could not be made in duplicate. The result of the pre-exposure is clear, but not great (fig. 1).

From this experiment we may get the impression that pre-exposure

increases both the uptake in the dark and the one in the light, but the inaccuracy of the observations with uptake in the dark does not permit us to consider it as proved. In order to get clearer data on the effects of pre-exposure we need material that possesses the substance formed

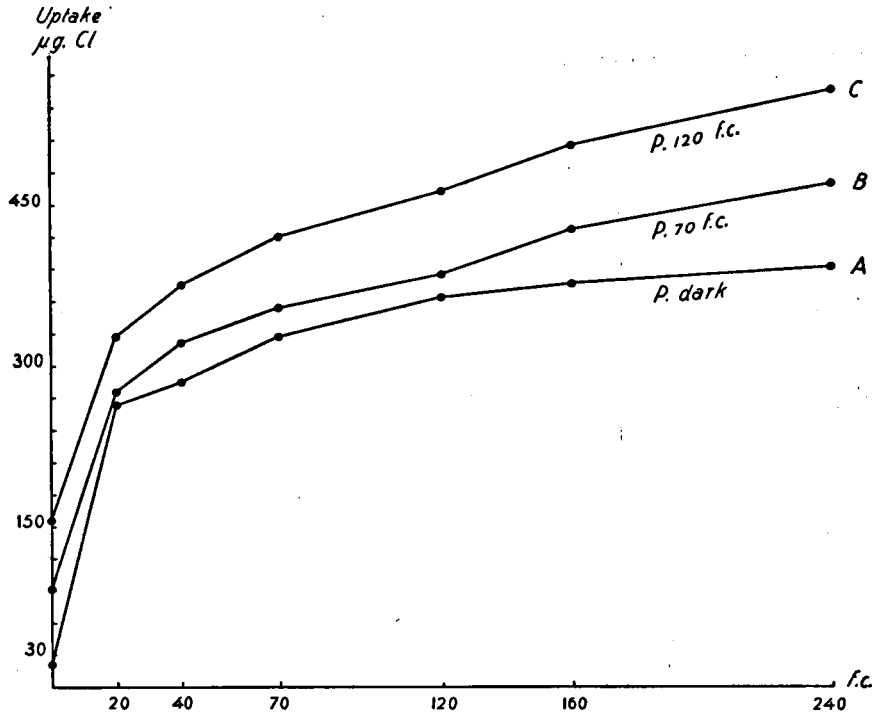

Fig. 1. Influence of exposure during the pretreatment on the uptake in different light intensities (Sodium light). Pretreatment: A. in the dark; B. in 70 f.c.; C. in 120 f.c., 24 hours. Uptake from 1 mM KCl + $CaSO_4$ solution. Aeration with air — CO_2. Abscissa light int. in foot candles. Ordinate uptake in µg Cl′ per series of 8 leafsegments (length 2.5 cm, width 0.4 cm) 25°C, 24 hours. Exp. 1098

in the light only in a slight degree before the experiment. A prolonged preceding dark period works in this direction.

In 1947 it had been investigated whether the influence of pre-exposure is only active during the first hours of uptake or that the influence of pre-exposure is perceptible during the entire uptake period. For this investigation some two experiments had been made, in which after pretreatment in the dark, at 70 f.c. and at 150 f.c. the uptake had been determined after 6 and after 24 hours (corrected statement). It was ascertained whether the effect of light during the pretreatment influenced the uptake only in the next few hours following the pre-treatment or that it lasted for a longer time. The results are given in fig. 2, which contains the data from Table 7 of ARISZ's paper (1947).

It appears that through pre-exposure the rate of uptake increases in the first 6 hours as well as in the next following 18 hours. From

these experiments Arisz drew the conclusion that during the pre-treatment in light a substance e.g. a sensitizer may be formed which favours the process of uptake in the light. In order to ascertain whether a sensitizer was really formed, these experiments have been repeated

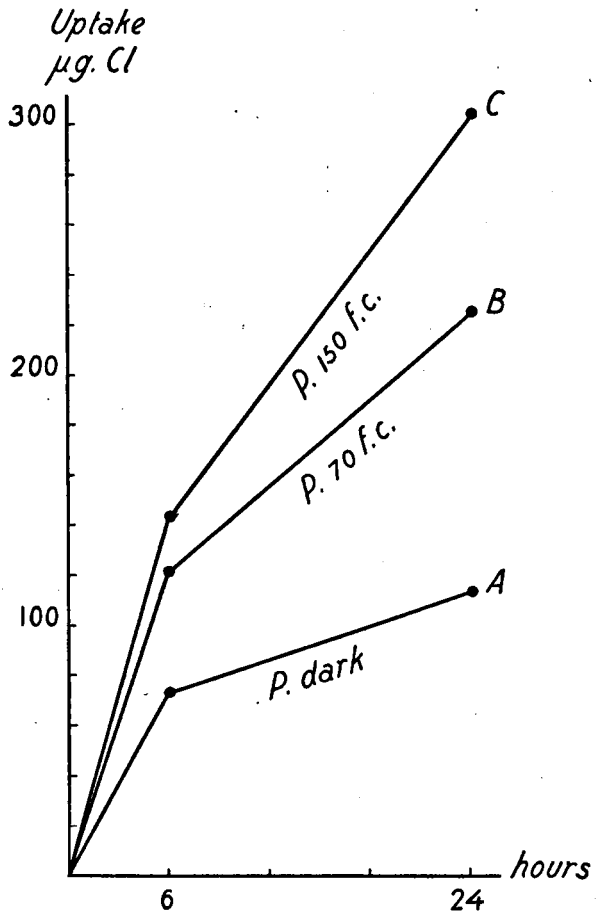

Fig. 2. Influence of exposure to light during the pretreatment on the uptake of 1 mM KCl + $CaSO_4$ solution, 24 hours, 25°C. The uptake is determined after 6 and 24 hours. A. pretreated in the dark; B. pretreated in 70 f.c.; C. pretreated in 150 f.c. Abscissa time. Ordinate uptake in µg CL'.

with strict precautions. In 1947 we had omitted to investigate whether through pre-exposure the uptake in the dark was increased as well. This is comprehensible, because the uptake in the dark for the material then cultivated in daylight was too small for such determinations to be made. In fig. 1, however, distinct differences were found for the uptake in the dark.

Fig. 3 contains the result of experiment 1107 on the uptake after

4, 8 and 24 hours in the dark and at 50 f.c., of material pretreated either in the dark or at 50 f.c. or at 150 f.c.

To obtain a clear effect the material was put in the dark for 24 hours, before the proper pretreatment was started. The result of this experiment is satisfactory. All determinations have been made in duplicate.

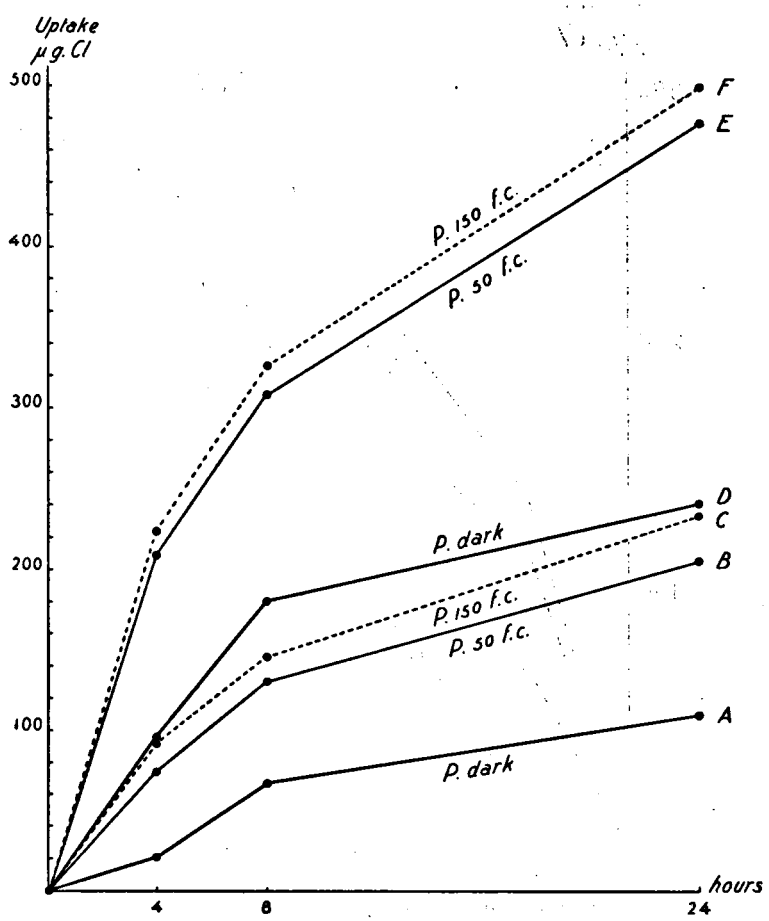

Fig. 3. Influence of exposure during the pretreatment on the course of the chloride uptake. A and D pretreatment in the dark 24 hours. B and E in 50 f.c. C and F in 150 f.c. A, B, C uptake in the dark, D, E, F, uptake in 50 f.c. 24 hours, 25°C. The uptake is determined after 4, 8 and 24 hours. Experiment 1107.

From the three lower lines A, B and C it appears that also with uptake in the dark the nature of the pretreatment has its influence. The line for pretreatment with 50 f.c. lies distinctly higher than the one for pretreatment in the dark and the one for 150 f.c. lies still higher. Besides the slope of the lines both in the first and in the second period increases distinctly after stronger pre-exposure. In the third

period the difference is still visible as well. The same thing holds good for the three upper lines D, E and F, for which the uptake at 150 f.c. was examined. Here the differences in slope between the leaflengths which have been pretreated in the dark and those exposed to 50 f.c. are much greater, but the difference with 150 f.c. pretreatment is small, because at this light intensity light saturation has been reached.

This experiment supports the previous experience that the influence of pre-exposure is demonstrable during the entire period of uptake. What is new, is that the influence of pre-exposure is also demonstrable with uptake in the dark. We must therefore assume that pre-exposure does not form a sensitizer or that the concentration of chlorophyll increases, but that a substance is formed which increases both the uptake in the dark and the uptake in the light. We may add that in this experiment too it has been our object to have exposure take place in a CO_2 free medium.

Seeing it was already demonstrated above that the substance formed in the light is transportable, it seems possible that this substance deserves the name of carrier.

We now revert to Table 15, from which it appeared that sucrose addition during pretreatment also increases the uptake. This influence of sucrose on the uptake in the light at 50 f.c. has been verified for an uptake of 4 hours, 8 hours and 24 hours (fig. 4). It appears from experiment 1106, that pretreatment with sucrose increases the uptake for the whole duration. So here too a substance is formed during the pretreatment which is itself not consumed in the uptake, so that the action diminishes, but which gives a consistently higher uptake of chloride. A few experiments originally gave us great pains with the interpretation. In these pre-exposure had but a slight effect. So in experiments 1097 and 1103 (Table 17) it makes no difference whether pretreatment takes place in the dark at 10 f.c., at 40 or at 70 f.c. An addition, however, of 1/20 M sucrose during the pretreatment distinctly raises the uptake in all cases. The effect even appears to be greater

TABLE 17

Influence of pretreatment on the uptake of chloride in the dark. Pretreatment 24 hours in the dark and in 10, 40 and 70 f.c. In A chloride uptake in the dark (24 hours), in B chloride uptake in the dark with sucrose 0.05 M, in C chloride uptake in the dark. Here sucrose was added during the pretreatment. Exp. 1097 and 1103.

pretreatment	A uptake dark		B uptake dark with 0.05 M sucrose		C uptake dark after pretreatment with 0.05 M sucrose	
	Exp. 1097	1103	Exp. 1097	1103	Exp. 1097	1103
dark	64	32	103	156	167	209
10 f.c.	60	32	110	153	167	199
40 f.c.	57	39	103	153	167	219
70 f.c.	60	43	103	156	163	216

than, when sucrose is administered during the uptake. It is clear now that in this case material has been used which was rich in 'light substance' and poorer in 'sugar substance' so that light did not have any influence during the pretreatment and sugar did.

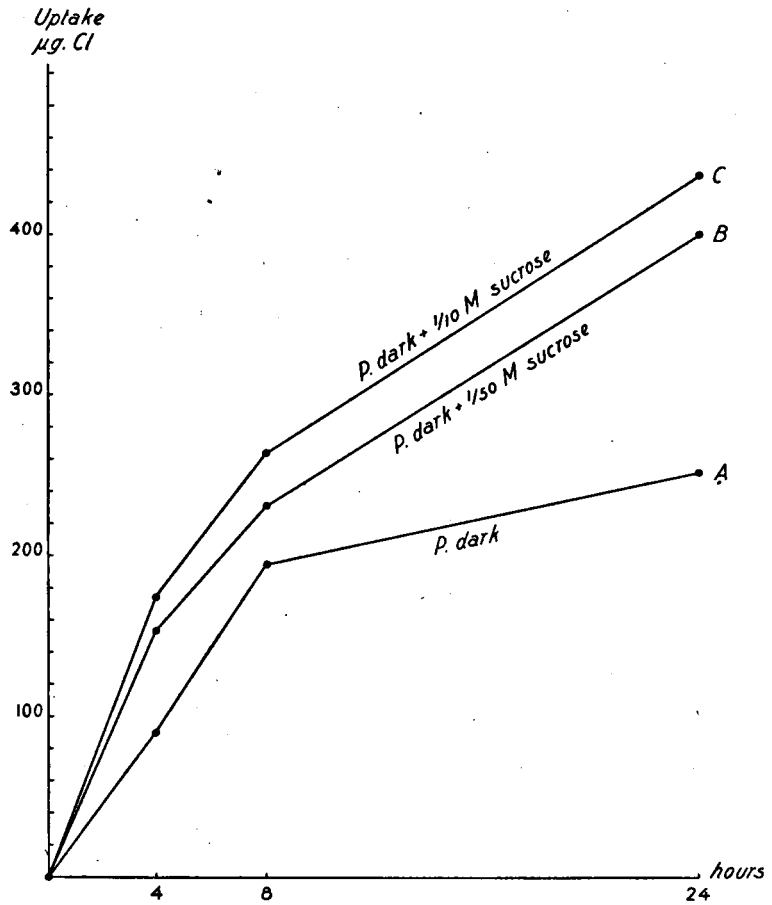

Fig. 4. Influence of supply of sucrose during the pretreatment on the course of the chloride uptake. A pretreated in the dark, B in the dark + 1/50 M sucrose, C in the dark + 1/10 M sucrose. Uptake: 50 f.c. in absence of CO_2, from 1 mM KCl + $CaSO_4$ solution, 25°C. The uptake is determined after 4, 8 and 24 hours. Experiment 1108.

In experiment 1090 (Table 18) the same was found. Here pre-exposure to 150 f.c. without CO_2 gives the same results as pretreatment in the dark, but sugar addition to the free part strengthens accumulation both in the absorbing and in the free part. These results also indicate that pre-exposure and supply with sugar during the pretreatment bring about two effects which occur independent of each other.

TABLE 18

Influence of pretreatment in the dark and in the light on the uptake of chloride from 0.01 M KCl + $CaSO_4$ added to agar strips (exp. 1090). In this case there is no difference visible.

Uptake	pretreatment in the dark	pretreatment in 150 f.c. — CO_2
in the dark	217	202
150 f.c. — CO_2	373	383
dark — dark	181 — 67 (248)	188 — 82 (270)
dark — light	245 — 153 (398)	270 — 174 (444)
dark — light + 0.05 M sucr.	323 — 227 (550)	340 — 252 (592)

Discussion

From the experiments on the influence of sucrose and light on the uptake and transport of chlorides in *Vallisneria* leaves that have been discussed here, it appears that it is possible to make considerable transports of chlorides take place and to examine them quantitatively. The great uptake in these experiments is due to the cultivation of the Vallisneria plants in artificial light in soil poor in chloride and in water from which the salt has been removed by ion exchangers. Dependent on exposure and administration of sucrose 0-500 μg Cl is taken up per series of 8 leaf segments of 2.5 cms. Most Cl is stored in the vacuoles as may be inferred from the increase of osmotic value. In the discussion of the results we have started from the symplasm theory that the ions are first taken up into the symplasm, next carried in the symplasm from cell to cell, after which there is a secretion of ions from the symplasm into the vacuoles. In *Vallisneria* leaves the mitochondria are not yet sufficiently investigated to be discussed in this paper. As carriers for intercellular transport they seem to be unsuited because they cannot migrate from cell to cell. They may have an important influence on uptake processes by providing energy or they may function as carriers in the cell cytoplasm as suggested by Robertson.

In the interpretation of the transport experiments various difficulties present themselves. It may be imagined that a transport in the leaves of Vallisneria makes use of various courses. Starting from previous results, it was found (Arisz 1947, 1948, 1953) that the symplasm is a path for transport of salts; besides we can imagine a transport through the cell walls and through the bundles in Vallisneria. It is difficult to decide whether in certain cases the cell walls act a part. In addition to the arguments already given in previous publications which point to a plasmatic transport, the influence of illuminating the absorbing zone adds a fresh argument. For does not exposure of the absorbing part render it possible that accumulation both in the absorbing and in the free zone greatly increases? As long as the absorbing zone is in the dark, the exposed free part can accumulate but small quantities of chloride. If there was a considerable uptake of chloride ions by the cells of the free part from the surrounding cell walls, the exposure of the absorbing part could not have such a great influence.

The weak transport in some experiments (e.g. experiments 1024 and 1088) shows the slight significance of the cell walls compared with the symplasm. It cannot be assumed that if the cell walls could transport ions, this process would be so variable. For a symplasm connected by the sensitive plasmodesmata, however, these differences in conductivity can be understood (ARISZ and SCHREUDER 1956). As a cause of transport in cell walls especially differences in suction tension are to be considered. In the submerged leaves of Vallisneria they do not act a part, so only diffusion in the wall capillaries would be left.

The significance of the bundles for transport will not be discussed here. We may refer to a recent paper (ARISZ and SCHREUDER 1956), from which it appears that the bundles of *Vallisneria* transport chloride, probably in a somewhat stronger degree than parenchyma cells. ARISZ (1952) considered the sieve tubes as tracts of specialised cells, which at least during part of their life transport substances in the same way as parenchyma cells do (cf. also SCHUMACHER und HÜLLSBRUCH 1955). Just as the parenchyma cells they form part of the symplasm.

We will begin with the influence of sucrose addition as it is easier to analyse than that of exposure. The sucrose is taken up into the leaf cells and converted by metabolism. Of course the supply of carbohydrates in the leaflengths with which the experiments are made, shows a great divergence owing to the different previous history and consequently a uniform reaction cannot be expected in experiments made at various times. The general impression of the influence of administering sucrose is a local stimulation of the accumulation. The effect is more localised than that of exposure, though transport of sugars certainly takes place. The favourable influence of sucrose often appears only on the spot of administration. If it is administered to the absorbing part in the dark, accumulation in the absorbing part increases, whereas it may decrease in the free part at the same time. This indicates competition of the secretion from the symplasm in the absorbing part with that in the free part. As a rule the total uptake increases in this case. By exposure and local administration of sucrose this competition may be controlled, but the unknown transport factor determining the conductivity for ions of the symplasm, will also influence the development of this competition. If, however, the absorbing zone is in the light and there are more chloride ions present in the symplasm, there is no competition since the ion supply is not limiting any more. In some cases the accumulation in the free part is then increased too.

Sucrose administered to the free part, the absorbing part being exposed to light, usually also gives a local increase of the secretion in the free part. Particularly if the absorbing zone is in the dark, the osmotic long distance phenomenon already mentioned, which can greatly reduce the accumulation in the absorbing part, appears.

This phenomenon is connected with a decrease in conductivity owing to dehydration of the symplasm (ARISZ and SCHREUDER 1956). As in the absorbing part the peripheral cells take up the chloride ions

direct from the medium and introduce them into the symplasm, the other cells will have to obtain the chloride ions through the symplasm. If, therefore the conductivity has been diminished by a suction tension, this will also make itself felt in the absorbing part. To the free part which is in touch with the sucrose and which will be able to take it up, some chloride will be carried, which may in some cases lead to considerable accumulation of chloride in the free part.

Sucrose administered during pretreatment has a stronger influence on the accumulation than if it is administered during the uptake (Table 17). It raises the rate of accumulation during several hours, during which sucrose is equally effective on uptake in the dark as in the light.

Summing up we may say that sucrose increases accumulation locally and that this may be attended by a higher uptake of ions from the medium. This must be based on a specific influence of the sugar on the absorption of ions from the outer solution or on a promotion of the secretion process, which accumulates ions from the symplasm into the vacuoles.

The local effect of sugar administration and the competitive effect between accumulation in the absorbing and the free part point to the last mentioned process.

It is conceivable that if the "membrane" as various investigators (MEYER, TEORELL, FREY WIJSSLING, VERVELDE, ARISZ) assume is a Donnan system a certain relation between the chloride ion concentration in the medium and in the symplasm is being maintained, so that by diffusion of ions from the medium the concentration in the symplasm is restored, whenever it decreases by secretion of chloride ions into the vacuoles. In this way the secretion of ions into the vacuole can regulate the ion uptake from the medium. Still the rate of ion uptake into the symplasm in the dark is limited in the presence of sucrose and cannot rise above a certain level.

The influence of sugar on the uptake has been explained here by strengthening of the accumulation process, i.e. the secretion of chlorides from the symplasm into the vacuoles. We have now to consider if the symplasm of *Vallisneria* leaves is a free space in the sense of ROBERTSON, HOPE, BUTLER, EPSTEIN, BURSTRÖM and LUNDEGÅRDH, which is in communication with the free space in the walls and with the medium. The rate of ion uptake into the plasmatic free space must, however, be under the influence of the 'membrane', which is considered a Donnan system in this case. With regard to the symplasm we might speak of a restricted free space.

Difficulties arise, however, for the theory of free space, when the light effect is treated. For when the free part is exposed, the absorbing part being in the dark, the rate of uptake cannot exceed a certain limit.

By exposure of the absorbing part, however, the rate increases considerably, so that also the free part accumulates more chloride ions. From this it appears, as has already been discussed, that the supply of chloride ions to the free zone does not take place in the wall free space without the intermediary of the symplasm of the absorbing

zone. It also follows from this that light affects the permeation of ions into the symplasm, i.e. that under the influence of light a process takes place which causes more chloride ions to be absorbed into the symplasm. The greater availability of chloride ions in the symplasm cannot be due to the influence of light on the accumulation in the vacuole. Primary is the greater availability of chloride ions in the symplasm and secondary is the stronger secretion into the vacuoles of absorbing and free parts.

Experiments have been performed to demonstrate the presence of a free space in *Vallisneria* leaves. In Table 19 some data are given on

TABLE 19

Experiment on the presence of an apparent free space in Vallisneria leaves. After uptake of chloride from solutions of KCl of different concentrations during 24 hours the first series is analysed after rapidly rinsing in distilled water, the second series is rinsed in the dark during 15 minutes in aerated distilled water, the third series during 30 minutes. There is no loss at all of absorbed chloride ions. Experiment 1113.

	rapidly	15 minutes	30 minutes
after 24 hours' uptake of 0.00025 M KCl rinsed	259	263	266
after 24 hours' uptake of 0.001 M KCl rinsed	316	323	316
after 24 hours' uptake of 0.004 M KCl rinsed	359	362	355
after 24 hours' uptake of 0.016 M KCl rinsed	408	415	415

the uptake of different concentrations of KCl in the light and a subsequent loss of chloride ions by leakage in aerated distilled water in the dark. A loss of chloride ions from the tissue was not found. Likewise leaf segments exposed after uptake to light in distilled water during one hour showed no loss of chloride ions. Therefore the presence of a free space for chloride ions cannot be shown in this way.

Light has a strong influence on the uptake of chlorides. In the presence of a concentration of carbon dioxide higher than that of normal air a considerable quantity of carbo-hydrates is formed. It is clear that if in the photosynthesis sugars are formed, they will promote the accumulation of chlorides in the same way as has been found when sucrose was added.

Table 20 gives data about the influence of exposure to light during

TABLE 20

Influence of exposure to light during the pretreatment and the uptake in the presence or absence of carbondioxide. Light intensity 100 f.c. Uptake in μg Cl from a solution of 1/1000 M KCl + $CaSO_4$ after pretreatment: A in the light with CO_2, B in the light without CO_2, C in the dark with CO_2; duration of pretreatment and of uptake 24 hours; 25°C. Experiment S 96.

Pretreatment	uptake	μg Cl
A Light + CO_2	light + CO_2	547
	light — CO_2	523
B Light — CO_2	light + CO_2	403
	light — CO_2	410
C Dark + CO_2	light + CO_2	303
	light — CO_2	165

the pretreatment and the uptake in the presence or absence of carbon dioxide. Aeration with carbon dioxide containing air during the pretreatment has the same effect on uptake as exposure to light with addition of sugar. The experiment shows the double influence of exposure during the pretreatment, viz, the formation of a specific "light substance" which increases the following uptake and the formation of sugar in the presence of carbon dioxide. With this material exposure during uptake to light in the presence of carbon dioxide had only influence after a pretreatment in the dark.

It is evident that light also has a more specific influence on the uptake process, as both a previous exposure and an exposure during the uptake in a medium free from carbon dioxide greatly promotes the uptake. VAN LOOKEREN CAMPAGNE (1956) found that the action spectrum of the photosynthesis in light of various wavelengths perfectly corresponds with that of the photo-accumulation of chlorides. There is a difference since the saturation for the chloride uptake was found at a lower light intensity than the saturation for photosynthesis. As presence of carbon dioxide is not required for photo-accumulation, the common feature of photosynthesis and photo-accumulation is based on the absorption and the transference of light energy by the chlorophyll. In photosynthesis this energy is used for the reduction of substances that have incorporated atmospheric carbon dioxide, in photo-accumulation for processes that cause uptake and accumulation of chlorides. It might therefore be imagined that light and sucrose would influence the uptake process in an identical way, e.g. as a result of formation of an energy rich substance both in photosynthesis and in respiration. This, however, does not seem to be the case, for even with light intensities at which uptake of chloride is maximal, a considerable increase in uptake of chloride may be caused by addition of sucrose. (Table 4). On the ground of van Lookeren Campagne's data it may be expected that the formation of an energy rich substance will be continued also at higher light intensities than at which salt uptake is maximal, so that at light saturation a dark reaction becomes limiting for the uptake. Therefore sugar cannot be active through the formation of the same substance which is formed in the light in the absence of carbon dioxide but sugar must influence the dark reaction.

Four light effects have been studied.

1. A previous exposure in carbon dioxide free medium increases the rate of chloride uptake. A substance is formed which is transportable. If an adjoining part of the exposed leaflength remains in the dark during the pretreatment it receives part of the substance formed in the exposed adjoining part, so that a subsequent chloride uptake is increased (Table 16). This substance promotes the chloride uptake as well in the dark as in the light. Therefore it is not a sensitizer but a kind of catalyst or enzyme which plays a part in the uptake. It does not seem to be readily consumed by the uptake process as it remains effective during several hours.

2. Exposure to light during the uptake in carbon dioxide free medium

promotes the uptake. The effect is dependent on the intensity of the light. The uptake may go on during many hours at the same rate or the rate may diminish after a longer or shorter time. Up to now there is no indication that during the exposure a substance is formed which increases the rate of uptake. This may indicate that exposure during uptake does not produce the substance which is formed during the pretreatment in distilled water. It seems that this substance is only formed in the light when the medium is free from salt ions. If salt ions are present the light energy is used to take the ions into the symplasm.

3. In transport experiments an exposure of the absorbing part of a 5 cm leaflength causes an increase of accumulation both in the absorbing and in the free part. If light acted only locally, as sugar does on the accumulation in the absorbing part, we should be inclined to think of an influence of light on the secretion of the ions from the symplasm into the vacuoles.

Now that, however, the accumulation in the free part also increases, even if it is in the dark, this indicates an influence of the light on the availability of ions in the symplasm. Formerly it would have been called an influence of light on the permeability and it would have been interpreted as an influence on the size of the pores in the boundary surface of the protoplasm. Nowadays, however, we know that the uptake does not only depend on the membrane but on metabolic processes as well. The data are insufficient to give a further analysis of the way light acts on the uptake process.

4. Exposure of the free zone of a transporting leaflength has an effect on the accumulation in the whole symplasm. This means that a substance is produced in the exposed free part which is transferred to the absorbing part. This would be an interesting process if we were quite sure that the substance formed in the light in the free part of the leaflength is not a carbo-hydrate. It is rather difficult to give conclusive proof that this influence on the accumulation in the absorbing part is not the result of photosynthesis in the light. Still it is remarkable that this influence is distinct even if sucrose 1/20 M is administered to both parts of the leaflengths. The experiments have therefore been repeated in carbon dioxide free medium, so that at the most the carbon dioxide produced by respiration was available to be used in photosynthesis (Table 6 exp. 1112 and Table 7A exp. 1118). It seems unlikely that this extremely small quantity of carbohydrates can have caused the rather important effect on the accumulation in the absorbing zone.

The use of longer leaflengths of 7.5 cm gives the opportunity to separate the exposed free zone from the absorbing zone by an intermediary zone of 2.5 cm, which remains in series 1 and 2 in the dark and in series 3 and 4 in the dark with addition of sucrose (Table 21). The influence of an exposure to light of the third zone in series 2 on the uptake in the absorbing zone is rather great. Addition of sucrose to the second zone (series 3) increases uptake only slightly but exposure to light of the third zone even with simultaneous addition of sucrose

to the second zone (series 4) has a considerable influence on the accumulation in the absorbing zone. This is a strong indication that light forms other substances than sucrose in the free leaf zone which are moved in the symplasm and promote the uptake and the accumulation in the absorbing part. If this conclusion is justified it raises the

TABLE 21

Influence of exposure to light of a zone of the free part on the accumulation in the absorbing zone. Leaflengths of 7.5 cm are placed in perspex boxes divided in three compartments. The first zone of 2.5 cm is exposed to light and absorbs chloride from agar strips with 0.01 M KCl, $CaSO_4$ and 0.05 M sucrose. The second zone is in the dark in distilled water, series 3 and 4 with 0.05 M sucrose added. The third zone is between agar strips containing 0.05 M sucrose. This zone is in series 1 and 3 in the dark, in series 2 and 4 exposed to light. Pretreatment in distilled water exposed to light during 24 hours. After the 24 hours' uptake the leafzones are separated and the chloride content in μg Cl of 8 single segments is estimated. Experiment 1117.

series	first zone	second zone	third zone	chloride content
1	light + sucrose	— dark	— dark + sucrose	430 — 75 — 68
2	light + sucrose	— dark	— *light* + sucrose	**543** — 110 — 117
3	light + sucrose	— dark + sucrose	— dark + sucrose	444 — 89 — 75
4	light + sucrose	— dark + sucrose	— *light* + sucrose	**529** — 124 — 110

question whether the product formed in the free part in the light in the absence of carbon dioxide could be identical with that formed during the pretreatment in the light, which as we have seen is also transportable. At the present time our knowledge of these processes is not sufficient for us to give a satisfactory answer to this question.

SUMMARY

Sucrose and light have a regulating influence on the accumulation of chloride ions in the cells of *Vallisneria* leaves. The light effect is not only the result of the formation of carbo-hydrates in the photosynthesis, light also being active in the absence of carbon dioxide. Seeing that with light saturation for the chloride uptake sucrose still increases the uptake, the influence of light and sucrose is not due to the formation of the same substance.

Light promotes the uptake into the symplasm and as a result increases the transport in the symplasm and indirectly the secretion of chloride ions from the symplasm into the vacuoles. This obtains as much for the vacuoles in the absorbing part as for those in the free part of the leaf.

If the uptake takes place in the dark, it has a limiting influence on the accumulation in the exposed free part. This proves that the free part does not take up chloride ions direct from its surroundings. Therefore transport of chlorides through the cell walls to the free part does not take place.

Exposure of the free part, the absorbing zone being exposed to light, causes an increase of accumulation in the absorbing zone. This indicates that a substance is formed by the exposed free part and transported to the absorbing part which increases accumulation of chloride ions.

Exposure of the free part, the absorbing zone being in the dark with sucrose, may likewise result in an increase of the total uptake, so that the accumulation both in the absorbing and in the free part increases. If, however, the absorbing zone is in the dark without sugar, the effect is variable.

The influence of sucrose administered to the absorbing or to the free zone is in

the main local, though sometimes an increase in accumulation occurs in the adjoining part. This can be explained by transport of the absorbed sugar.

Sucrose administered to the absorbing part in the dark increases the secretion of chloride ions into the vacuoles in the zone of administration. This sometimes takes place at the expense of the accumulation in the free part. This is due to the fact that the uptake of chloride ions in the dark and in the dark with sucrose is limited, so that the absorbing part must compete with the free part to accumulate the ions into the vacuoles. The rate of transport in the symplasm and the presence of carbo-hydrates determine the result.

Sucrose in the free zone, if the absorbing zone is in the light, likewise gives a local increase of the secretion into the vacuole; in some cases the secretion into the absorbing part also increases. Addition of sucrose to the free part may have an osmotic effect on the absorbing part, owing to which it accumulates fewer chloride ions in the vacuoles. This influence may be compensated by giving sucrose to the absorbing part.

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