Acta Botanica Neerlandica 7 (1958) 635–653

THE INFLUENCE OF SALTS ON PROTOPLASMIC STREAMING GENERATED BY LIGHT IN SUB-EPIDERMAL CELLS OF VALLISNERIA LEAVES

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(*received July 11th, 1958*)

INTRODUCTION

Several investigators observed the occurrence of protoplasmic streaming in mesophyll cells of *Vallisneria spiralis* and used this object for studying the phenomenon of protoplasmic streaming either in connection with other physiological phenomena or not. FITTING (1925) and SCHWEICKERDT (1928) showed that light can initiate streaming. Even intensities of half a meter candle were effective. Red light produced the greatest effect, next blue. Green light had only a slight effect.

BEIKIRCH (1925) claimed that the stimulating effect of light on the streaming manifests itself as the initiation of streaming in quiescent cells. Light does not increase the rate of streaming in cells which showed already streaming. The change in the number of cells showing streaming and not the alterations in rate of streaming would give a measure for the effect of light.

The effect of various chemical substances on the initiation of protoplasmic streaming was investigated by FITTING (1925, 1928). In fact Fitting looked for the very active substance(s) occurring in aqueous extracts of *Vallisneria* leaves, which in very slight quantities caused streaming in quiescent cells. Further investigations on the effect of salts on protoplasmic streaming with other objects were made among others by SEIFRIZ (1922, 1923), CHOLODNYJ (1923), COLLA (1929) and SEIFRIZ and URAGUCHI (1941). The results of these researches varied strongly.

In this laboratory Miss M. Heikens found that 0.001 M KCl has an inhibiting effect on the initiation of protoplasmic streaming by light (see ARISZ 1956, p. 49). Normally exposure to light of *Vallisneria* leaf lengths in water induces streaming. The subjoined research gives data on the influence of various salts on the initiation of protoplasmic streaming by light.

MATERIAL AND METHOD

The material is partly taken from a cultivation tank in one of the hot-houses (experimental series I–V). For the next experimental series material has been used cultivated under constant conditions as to temperature and light in concrete tanks in the cellar.

Sound leaves are taken, cleared of epiphytic algae, and cut into lengths of 5–6 cms. These lengths are washed with glass-distilled water and next kept in the dark floating in this water. The water is renewed twice daily. During this period aeration takes place.

After a three days' pretreatment the cells are checked on the occurrence of protoplasmic streaming. For this purpose the leaflengths are transferred to petri-dishes containing water and fixed to the bottom of the dishes with tiny flat glass rods. The leaf length is used for further experiments when its cells do no more show any streaming. The water is sucked off and replaced by the appropriate salt solution. Thirty minutes after the salt solution has been added, it is ascertained whether there is a salt effect resulting in a streaming meanwhile set in. An hour after addition of the salt solution the leaf lengths are exposed to an incandescent lamp for ten minutes (light intensity on the leaf length about 55 foot candles). After waiting for another ten minutes, we determine the streaming percentage.

The phenomenon of protoplasmic streaming has been studied on subepidermal cells. These are more sensitive than the epidermal cells. The latter (at least in these experiments) do not give any light reaction. In those experiments in which the epidermal cells did react, this has been stated.

All figure material has relation to subepidermal cells. The movement of chloroplasts and nucleus serve as indicators for the occurrence of streaming. The type of streaming is rotation.

A leaf length lying in water shows streaming after exposure (light reaction). This streaming is most frequent about twenty minutes after the beginning of exposure and continues to be so for about forty-five minutes and decreases after that (Schweickerdt).

In experimental series (I–V) the behaviour of the leaf lengths treated with a salt solution has been compared with the behaviour of those treated in water. As the salt effect proved to be reversible, a different method was followed, in which one and the same leaf length was used for a number of experiments.

For microscopy a microscopical lamp with green filter is used, because green light has only a very slight influence on the light reaction with *Vallisneria* (Fitting, Schweickerdt). The water-immersion objective did not produce streaming as a result of loss of ions to the liquid. The temperature of the dark room is kept equal to that of the cultivation tanks (23° C).

As a measure for the plasmatic streaming initiated, the streaming percentage was determined by counting a hundred cells, i.e. five groups of twenty, distributed at random over the leaf length. From these the number of cells showing streaming, has been kept as streaming percentage. Though not very accurate, this method is quite usable for obtaining intercomparable values.

Unless otherwise stated, the experiments are made during the winter season.

RESULTS

1. *Influence of* 0.001 *and* 0.01 M KCl *on the light reaction*

The streaming percentages after treatment with two different KCl concentrations as compared with the streaming percentages after treatment with water, are given in table I. The inhibiting effect of these KCl concentrations on the light reaction is clear.

Each of the streaming percentages mentioned has been obtained from a different leaf length. Each leaf length has been used only once. The computed averages have all been rounded off to whole numbers; so has the mean error.

TABLE I

The effect of KCl on the light reaction in *Vallisneria* leaves. The streaming percentage was determined after a ten minutes' exposure in leaf lengths which were kept either in water or in KCl solutions

Streaming percentage in:		
water	KCl 0.001 M	KCl 0.01 M
%	%	%
16	0	0
12	0	3
10	4	4
45	0	0
32	10	0
28	3	3
39	2	0
29	1	0
46	0	2
10	1	4
32	8	1
36	0	0
22	0	0
65	0	4
90	0	3
11	0	3
14	0	0
30	3	2
13	3	0
72	1	0
54	1	3
37	0	-
33 ± 5	2 ± 1	2 ± 1

2. *Influence of* KCl + $CaSO_4$ *on the light reaction*

Because so called "one salt solutions" often show toxic effects the above experiment has been repeated with KCl solutions to which $CaSO_4$ has been added. The ratio of weights KCl : $CaSO_4$ amounts to about 2 : 1.

As appears from table II the results are equal to those obtained with pure KCl solutions. Just as in table I a great variability appears to exist here in the streaming percentages of the leaf lengths in water.

Under the influence of KCl or KCl + $CaSO_4$ the streaming percentages are reduced to about equal low values.

TABLE II

The effect of KCl + $CaSO_4$ on the light reaction in *Vallisneria* leaves. The streaming percentage was determined after a ten minutes' exposure in leaf lengths which were kept either in water or in solutions of KCl + $CaSO_4$

Streaming percentage in:

water	0.001 M KCl + $CaSO_4$	0.01 M KCl + $CaSO_4$
%	%	%
53	0	1
46	1	1
58	0	0
25	0	0
81	2	2
22	2	3
21	0	0
44	0	1
50	3	0
39	0	3
65	1	4
72	1	1
46 ± 6	2 ± 1	2 ± 1

3. *Influence of* KCl *on streaming protoplasts*

From the above it appears that the initiation of protoplasmic streaming by light is strongly inhibited by treatment of a quiescent protoplast in the dark with KCl solutions at a concentration of 0.001 and 0.01 M. Is this inhibiting effect also shown in a streaming in progress, i.e. does a streaming protoplast settle sooner in a KCl solution than in water?

The experiments have been made as follows: streaming is initiated by a ten minutes' exposure to light. Next the streaming percentage is determined. Part of the leaf lengths remains in water; for the others

TABLE III

The effect of KCl on the length of the period of protoplasmic streaming in *Vallisneria* leaf lengths in the dark. The streaming was aroused by a ten minutes' exposure of the leaf lengths in water, the streaming percentage estimated and a part of the leaf length were transferred to the KCl solutions. From this moment the length of the period of protoplasmic streaming was measured

Period of streaming determined in:

water		0.001 M KCl		0.01 M KCl	
str. perc. at the start	length of the period	str. perc. at the start	length of the period	str. perc. at the start	length of the period
%		%		%	
36	140′	40	120′	35	95′
47	90′	53	120′	54	120′
20	110′	53	125′	55	145′
20	110′	19	135′	28	145′
42	100′	20	135′	60	120′

the water is replaced by the KCl solution. The length of the period during which the streaming again settles, is determined (table III).

It appears from the above that the period of streaming in KCl solution is not shorter than in water. From experiments carried out in the light in which there was exposure during the whole experiment something similar appears. The KCl solution was added immediately before exposure so that it cannot have had any inhibiting effect on the initiation of streaming in the light. In table IV the streaming

TABLE IV

The effect of KCl on the length of the period of protoplasmic streaming in *Vallisneria* leaf lengths in the light. The KCl solutions were administered immediately before exposure. The streaming percentages were estimated at different moments after the beginning of a continued exposure to light

Streaming percentage determined in:		
water	KCl 0.001 M	KCl 0.01 M
43 % after 17 min	42 % after 24 min	36 % after 35 min
22 % after 4½ hours	19 % after 4 h. 35 min	11 % after 5 h. 12 min
12 % after 30 min	10 % after 23 min	29 % after 42 min
40 % after 5 hours	37 % after 5 hours	48 % after 5 hours
7 % after 8 h. 35 min	20 % after 8½ hours	26 % after 8 h. 50 min
2 % after 24 hours	5 % after 24 hours	5 % after 24 hours

percentages have been given at definite moments after exposure was started.

It appears that the KCl at the concentrations used does not produce an inhibiting effect on a streaming protoplast. So KCl inhibits the initiation of the protoplasmic streaming by light but it has hardly any influence on the settling of the streaming.

4. *Reversibility*

The strong influence of the KCl concentrations used on quiescent protoplasts in the dark gave rise to the question whether the effects are reversible or not. It appears that the influence of the KCl concentrations can be entirely cancelled by "washing out" in water.

After a stay in KCl or KCl + $CaSO_4$ solutions for about two hours, the leaf lengths are transferred to water; this is renewed after about twenty minutes. It is then traced whether after a certain lapse of time, the light effect on the protoplasmic streaming has reached its original level. For leaf lengths which have been treated with 0.001 M KCl or KCl + $CaSO_4$ for two hours, a subsequent water treatment of about two hours proved to be sufficient.

In case of a 0.01 M KCl or KCl + $CaSO_4$ solution a longer time of water treatment (5–6 hours) is required. Szücs (1913) also found for this material that the effect of a previous treatment in aluminium salts could be cancelled by means of a "washing out" in water.

In the following experimental series this reversibility has been made use of, by using the same leaf length several times running. Between two successive experiments the leaf lengths are washed in water in their petri dishes.

5. *Influence of different anions*

In order to trace whether the influence of salts, when acting on the quiescent protoplast in the dark and the subsequent light reaction, is due to the anions or to the cations or to both, different K salts have been used in the following experimental series. If there should be an anion influence perceptible by this method, it will result in different values for the light reaction.

All salts were allowed to act at a concentration of 0.001 M on the quiescent protoplasts for one hour. In these series the light reaction has first been determined in water. All leaf lengths showing too low a streaming percentage, e.g. 25 % or lower, were discarded.

TABLE V

The effect of K_2SO_4 on the light reaction in *Vallisneria* leaves. One and the same leaf length is used several times. The values of the streaming percentages, it gave under the different circumstances, are put in a column. The streaming percentages are determined in water or in 0.001 M K_2SO_4 solutions

	Streaming percentages as given by the different leaf lengths:							
	%	%	%	%	%	%	%	%
water	44	45	69	58	52	39	64	52
K_2SO_4 sol.	3	3	4	4	1	1	2	1
water	39	44	—	42	24	12	19	22
K_2SO_4 sol.	4	3	10	4	—	—	—	—
water	38	34	36	22	—	—	—	—

Next we waited till the streaming had settled again, after which the light reaction under the influence of the salt used was determined. Then the leaf length was put in water till the salt effect had disappeared. Next another determination was made. The values of the streaming percentages (the light reaction) obtained in this way have been put the one below the other in the subjoined tables. (Thus the columns obtained show the behaviour of the same leaf length under different

TABLE VI

The effect of KNO_3 on the light reaction in *Vallisneria* leaves (0.001 M)

	Streaming percentages as given by the different leaf lengths										
	%	%	%	%	%	%	%	%	%	%	%
water	39	37	37	35	41	30	28	—	56	49	61
KNO_3 sol.	3	0	4	10	0	5	1	—	1	6	0
KNO_3 sol.	3	0	1	1	0	0	0	1	—	—	—
water	38	37	25	41	—	—	—	52	53	56	35

TABLE VII

The effect of KH_2PO_4 on the light reaction in *Vallisneria* leaves. (0.001 M)

	Streaming percentages as given by the different leaf lengths									
	%	%	%	%	%	%	%	%	%	%
water	64	21	41	38	46	54	58	75	56	50
KH_2PO_4 sol.	7	1	2	3	8	2	5	2	8	4
KH_2PO_4 sol.	2	0	2	0	3	0	3	1	0	—
water	43	21	12	15	—	2	16	0	2	48

salt conditions). In the subjoined tables some results have been rendered.

The influence of KCNS has been investigated a considerable time later. The reactivity of the material was then higher than in the experiments described above. The influence of KCNS deviates strongly from those of the other potassium salts. Already in the dark streaming is caused after half an hour. The effect of the KCNS treatment continues for a long time in water. The streaming does not settle until after more than 24 hours. The material seems to be in a fairly good condition after a KCNS treatment. Visible injuries have not been observed. Also the epidermal cells which normally continue being in rest show streaming under the influence of KCNS.

TABLE VIII

The effect of $KHCO_3$ on the light reaction in *Vallisneria* leaves. (0.001 M)

	Streaming percentages as given by the different leaf lengths						
	%	%	%	%	%	%	%
water	52	48	26	33	46	22	37
$KHCO_3$ sol.	2	5	6	4	6	4	5
water	36	48	24	30	42	26	33

TABLE IX

The influence of KCNS on the initiation of the protoplasmic streaming. The streaming initiated in the dark is estimated 30–60 minutes after the addition of the KCNS solution. (0.001 M)

	Streaming percentages as given by the different leaf lengths									
	%	%	%	%	%	%	%	%	%	%
KCNS dark	79	10	64	5	79	29	79	30	29	42
after exp.	85	22	61	42	84	47	71	59	70	59
KCNS dark	—	1	—	28	—	22	—	—	—	—
after exp.	—	27	—	82	—	46	—	—	—	—
water	39	—	81	0	43	0	20	45	77	—

If we compare the results of the experiments, it appears that most potassium salts at a conc. of 0.001 M inhibit the light reaction. The average streaming percentages were the following:

KCl : 4 ± 1	KH_2PO_4: 5 ± 1	KCNS d: 35 ± 7
KNO_3: 4 ± 1	$KHCO_3$: 4 ± 1	l: 54 ± 5
K_2SO_4: 4 ± 1	(all values rounded off to whole numbers)	

The influence of most potassium salts, i.e. chloride, nitrate, sulphate, biphosphate and bicarbonate on the initiation of protoplasmic streaming through light is the same. So it can be concluded that the result is a potassium effect. KCNS, however, gives much deviating results. These must be mainly due to the CNSion.

In order to trace whether the effect of salts on the initiation of protoplasmic streaming through light is a predominant cation or anion effect, the influence of various cations, in the presence of the same accompanying anion has been studied in the following series.

6. *Influence of different cations*

Also in these experiments, if possible, a leaf length has been used several times in order to investigate the influence of the various salts. The effect of the various salts has been determined ½–1 hour after addition of the salt solution in the dark and then after exposure. Some experimental series have been rendered in the subjoined tables.

TABLE X

The effects of 0.001 M KCl and RbCl solutions and a half strength Hoagland solution on the light reaction in *Vallisneria* leaves

	Streaming percentages as given by the leaf lengths										
	%	%	%	%	%	%	%	%	%	%	%
water	30	36	25	66	54	48	62	76	80	69	49
KCl sol.	6	5	1	4	4	3	4	2	4	—	—
RbCl sol.	5	4	1	1	0	2	4	0	2	0	0
Hoagland	11	7	2	3	4	2	2	6	7	2	3
water	29	27	13	—	—	—	—	18	17	69	11

The concentrations of the salt solutions used were all 0.001 M.

Monovalent cations. The inhibiting effects of KCl and RbCl on the light reaction are given in table X. Under the influence of NaCl a slight streaming occurs in the dark. Also the epidermal cells appear to show streaming. After transfer of the leaf length to water the effect of the NaCl is still perceptible as a streaming which continues for about 12 hours (See table XI).

TABLE XI

The effect of NaCl on the initiation of the protoplasmic streaming in *Vallisneria* leaves. (0.001 M NaCl)

	Streaming percentages as given by the leaf lengths in the dark (d) and after exposure (l)						
	%	%	%	%	%	%	%
water	30	41	58	—	—	—	—
NaCl sol. d	3	4	4	8	2	3	7
l	16	28	45	63	31	37	39
water	—	—	—	54	38	52	60
NaCl sol. d	—	—	—	0	0	2	1
l	—	—	—	26	24	40	25

TABLE XII

The effect of LiCl on the initiation of the protoplasmic streaming in *Vallisneria* leaves. (0.001 M LiCl)

	Streaming percentages										
	%	%	%	%	%	%	%	%	%	%	%
water	34	25	36	—	—	—	—	—	—	—	—
LiCl d	13	25	4	6	6	10	26	17	9	29	16
l	40	43	69	47	37	35	—	—	—	—	—

	%	%	%	%	%
LiCl d	10	12	15	12	13
l	74	86	54	78	68
water	77	69	25	77	59

In the dark LiCl gives a streaming which is fairly quick and continues long during the subsequent period in which the leaf lengths are floating on water. Streaming also occurs in the epidermal cells.

In summer the light reaction is stronger than in winter. The influence of the salt in the dark, however, is somewhat smaller in summer than in winter. The latter part of table XII gives values from experiments performed in the summer.

Divalent cations. The plant material, used for tracing the effects of Sr, had been picked six days ago. It floated on a sucrose solution from the third to the fifth day. This treatment induced some streaming. Therefore the leaf lengths were put back on water one day before the experiment proper, so that the streaming might settle.

Sr gives a fast and violent streaming in which the epidermal cells also take part (table XIII). After a 24 hours' water treatment the streaming is still intensive; by then, however, the leaf lengths are strongly infiltrated and contain a good many dead cells. The fact that Sr initiates streaming and injures the material had already been found by SEIFRIZ (1923) for *Elodea* and by FITTING (1928) for *Vallisneria.*

Under the influence of Mg streaming is initiated in the dark, in mesophyll cells as well as in epidermal cells. After exposure this number of streaming cells is increased considerably. This streaming continues for at least 24 hours in water. Even when the streaming has settled in water the influence of the Mg treatment is still perceptible in that after exposure to light a streaming is initiated which again lasts for a considerable period.

Injurious effects of Mg are hardly found. Fitting already found that $MgCl_2$ initiates the protoplasmic streaming in *Vallisneria* leaf cells. The experimental material was in the same condition as that of table XIII. Experiments performed in summer with $MgCl_2$ as well

TABLE XIII

The effect of $SrCl_2$ on the initiation of the protoplasmic streaming. (Conc. 0.001 M)

	Streaming percentages							
	%	%	%	%	%	%	%	%
water	66	60	53	55	73	43	53	49
$SrCl_2$ d	38	42	35	37	10	12	47	40
l	65	64	63	74	82	55	60	58

as $SrCl_2$ gave similar results. Fairly high values were obtained for the light reaction. The values for the streaming percentage in the dark were somewhat lower in summer.

In earlier experimental series data had been obtained on $CaSO_4$ and $CaCl_2$. Only a brief survey will be given below. Neither of the two salts gives streaming in the dark. On exposure both inhibit strongly the light reaction. The streaming percentages are reduced to the following low values under the influence of these salts:

$CaSO_4$:	5	7	0	5	0	3	2				
$CaCl_2$:	2	1	4	6	0	4	3	1	2	2	3

Trivalent cations. The data on the effect of Al are very few. Szücs (1913) had already found that Al salts initiate protoplasmic streaming in *Vallisneria.* Some injury of the material under the influence of aluminium sulphate occurs (table XV).

The influence of lanthanum chloride has been given in table XVI. Lanthanum chloride causes injury of the material, which perishes as a result of it. Consequently the streaming percentages are lower on exposure than in the dark. The same we find in a stronger degree for the

TABLE XIV

The effect of $MgCl_2$ on the initiation of the protoplasmic streaming. (Conc. 0.001 M)

	Streaming percentages				
	%	%	%	%	%
water	—	—	—	—	73
$MgCl_2$ d	25	56	38	27	15
l	61	61	71	80	85
water	18	30	52	46	—

TABLE XV

The effect of $Al_2(SO_4)_3$ on the initiation of the protoplasmic streaming (Conc. 0.0005 M)

	Streaming percentages			
	%	%	%	%
water	57	76	77	40
$Al_2(SO_4)_3$ d	30	5	20	40
l	46	24	59	45

TABLE XVI

The effect of $LaCl_3$ on the initiation of the protoplasmic streaming. (Conc. 0.001 M)

	Streaming percentages							
	%	%	%	%	%	%	%	%
$LaCl_3$ d	12	40	28	39	62	46	41	27
l	10	49	10	17	29	25	22	17

TABLE XVII

The effect of $Co(NH_3)_6Cl_3$ on the initiation of the protoplasmic streaming (Conc. 0.001 M)

	Streaming percentages							
	%	%	%	%	%	%	%	%
$Co(NH_3)_6Cl_3$ d	60	40	17	15	21	43	38	63
l	17	1	0	2	0	0	0	4

influence of hexammine cobaltichloride. In that case after two hours the material is already entirely limp and faded and perishes quickly. The plasm gets brownish and coagulates. At the beginning the epidermal cells show streaming; after a lapse of about two hours they perish too. Just as in the case of $LaCl_3$ the values of the streaming percentages are smaller after exposure than the corresponding values determined in the dark. The quicker deterioration of the material

under the influence of the hexammine cobalti ion is shown by the very low streaming percentages after exposure.

The average values of the streaming percentages obtained under the influence of the various salts, both in the dark (d) and after exposure (l) are rendered in table XVIII.

TABLE XVIII

Average streaming percentages (rounded off to whole numbers) with their mean errors, as obtained from subepidermal cells of *Vallisneria* leaves under the influence of the various salts

RbCl	d	0	$CaSO_4$	d	0	$Al_2(SO_4)_3$	d	23 ± 7
	l	$2 \pm \frac{1}{2}$	$CaCl_2$	l	3 ± 1		l	43 ± 7
KCl	d	0	$SrCl_2$	d	31 ± 7	$LaCl_3$	d	37 ± 5
	l	$4 \pm \frac{1}{2}$		l	64 ± 2		l	22 ± 5
NaCl	d	3 ± 1	$MgCl_2$	d	24 ± 7	$Co(NH_3)_6Cl_3$	d	37 ± 7
	l	34 ± 4		l	77 ± 5		l	3 ± 2
LiCl	d	14 ± 3						
	l	57 ± 6						

Clearly, the effects prove to differ and to be dependent on the cation. Within the series of monovalent cations there are distinct differences between the effects these cations have on the initiation of protoplasmic streaming. With the bivalent cations there is a great difference between the effect of calcium salts on the one side and magnesium and strontium chlorides on the other side. With the trivalent cations there are likewise differences. Under the influence of aluminum sulphate the leaf lengths show the highest streaming percentage after exposure, as in the case of mono- and divalent cations. Lanthanum chloride, but especially hexammine cobaltichloride gives after exposure the lowest values; they are injurious and cause the material to deteriorate after a short time.

On the whole it appears that those salts which initiate streaming in the dark do not or but little inhibit the light reaction, whereas the salts that do not initiate streaming in the dark greatly inhibit the light reaction.

7. *Influence of* KCl *on the initiation of the streaming by means of asparagine*

From Fitting's experiments (1928) it is known that the streaming can also be initiated by a treatment with asparagine in the dark instead of by a short exposure. In the following it has been traced whether KCl can also inhibit this asparagine effect. An asparagine concentration (0.001 M) was chosen, giving in a short time a frequent streaming. The leaf lengths have been pretreated for an hour with 0.001 M KCl. Then the light reaction was determined. Subsequently the KCl solution was replaced by a solution containing 0.001 M asparagine plus 0.001 M KCl. After an hour the streaming percentage was determined again. The results have been stated in table XIX.

From the above it appears that the differences between the streaming

percentages in experiments with KCl plus exposure and KCl + asparagine are but slight. In leaf lengths lying in a 0.001 M asparagine solution a considerable streaming is found after the lapse of an hour. (streaming percentages averagely about 50 %).

Therefore in this case the KCl likewise inhibits the initiation of protoplasmic streaming by asparagine.

TABLE XIX

The influence of KCl on the initiation of the streaming by asparagine as compared to the effect of KCl on the light reaction

	Streaming percentages			
	%	%	%	
KCl + exposure	0	2	1	
KCl + asparagine	0	8	14	
water + exp.	22	32	33	
KCl + asparagine	0	2	0	
	%	%	%	%
KCl + exposure	4	6	4	3
KCl + asparagine	10	10	16	3
water	—	—	—	—
KCl + exposure	2	0	0	0
KCl + asparagine	4	2	0	0
water + exp.	13	22	25	34

8. *Influence of sucrose on the initiation of protoplasmic streaming by light*

Protoplasmic streaming is a vital process the energy of which is produced by metabolism. Respiration is generally assumed to be the energy producing process. Just as respiration protoplasmic streaming is dependent on sugars and oxygen, the temperature being of influence. Experimentally made alterations, which affect respiration, also influence protoplasmic streaming (BOTTELIER 1934, OLSON and DU BUY 1940, THIMANN and SWEENEY 1937).

The Japanese investigators KAMIYA, NAKAJIMA and ABE (1957) conclude from their experiments with *Physarum polycephalum* that the streaming energy is provided by fermentation processes via ATP. Sugars are therefore a requisite for both ways of energy supply. The effect of sucrose on the light reaction has appeared from previous experiments.

If the leaf lengths had been used for a single experiment only a very slight streaming was observed during a subsequent check on the light reaction in water. A longer stay of the leaf lengths in regularly renewed water appeared not to increase the light reaction. Absence of this light reaction can possibly not be attributed to the ions left in the cytoplasm after the previous experiment (usually a few hours' stay in regularly renewed water suffices to re-introduce a good light reaction).

By floating the leaf lengths on a 0.1–0.01 M sucrose solution for 12–18 hours, a strong light reaction could again be obtained.

Leaf lengths which gave the following low values 0 %, 2 %, 0 %, 0 %, gave after floating on a sucrose solution for 12–18 hours 29 %,

59 %, 42 % and 44 % as streaming percentages respectively. After floating on the sucrose solution these leaf lengths floated on water for a considerable time. The favourable effect of a sucrose treatment continues for some time and remains still perceptible after experiments with salts. So after an experiment with monopotassium phosphate the following streaming percentages for the light reaction were obtained in water:

10 3 10 4 18 22 29.

Next the leaf lengths floated on a 0.1–0.01 M sucrose solution for more than twelve hours and during this treatment in the dark some streaming occurs again. The leaf lengths are then put in water till no perceptible streaming is found. Then an experiment with salts was made, which showed fairly normal inhibiting effects on the light reaction. In order to get rid of the effect of the salt a water treatment followed. Subsequently the following percentages for the light reaction in water were obtained (order of succession as in the above series):

45 41 29 30 76 80 39

The occurrence of streaming during the sucrose treatment may be the result of an increased supply of energy to the system of the streaming by the sucrose. It is, however, also possible that the viscosity of the cytoplasm is decreased by substances present in the sucrose. The occurrence of such substances and also of substances increasing viscosity, was demonstrated by STÅLFELT (1949) for very divergent plant material.

However, the fact, that the influence of the sugar addition continues to exist for such a considerable period of time in spite of the various water and salt treatments in this period, rather indicates that an increased energy supply as a result of the sucrose is the most important aspect.

DISCUSSION

From the experiments it appears that protoplasmic streaming occurs in quiescent subepidermal cells of *Vallisneria* leaves on exposure, when they are pretreated in water ("light reaction"). If a three days pretreatment is given with a KCl solution (0.001 M) or with a Hoagland solution in stead of water the light reaction does not occur. In intact leaves and also in excised leaves kept in the liquid of the cultivation tanks, very little, if any, streaming occurs. This was already known to HAUPTFLEISCH (1892) and FITTING (1925).

If we give to leaf lengths, kept in water, an additional one-hour treatment in 0.001 M solutions of KCl, RbCl or $CaCl_2$ no light reaction can be obtained. This inhibiting effect on the light reaction proves to disappear during a subsequent water treatment. This suggests that the presence of certain amounts of K and Ca in the plasm inhibits the light reaction (In 0.1 M solutions of KCl and $CaCl_2$, however, streaming is already initiated in the dark). During a subsequent treatment of the leaf lengths in water the cations present

in the plasm—adsorbed to negative groups—are removed in such a way—replaced by Hions—that the light reaction is again possible.

It is not possible that these cations are present in a "water-free space". In that case the cations would be washed out rapidly. The duration of the water treatment, however, to get again a sufficient light reaction is some hours.

When the influence of different potassium salts (conc. 0.001 M) is studied, the anions occurring normally in the medium of this plant, appear to show no effect of their own on the initiation of the protoplasmic streaming.

The identical effects found for these salts (KCl, KNO_3, $KHCO_3$, KH_3PO_4 and K_2SO_4) must be attributed to the potassium ion. The KCNS has a marked specific effect, which will have to be a CNS effect (fig. 1).

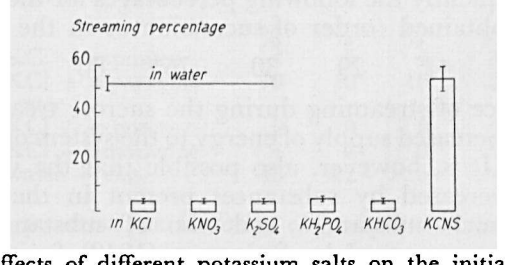

Fig. 1. The effects of different potassium salts on the initiation of the protoplasmic streaming by exposure to light (light reaction) in subepidermal cells of *Vallisneria* leaves. Salts as 0.001 M solutions in water added to leaf lengths with quiescent cells one hour before exposure to light. During ten minutes the leaf lengths were exposed to light of an incandescent lamp. As a measure for the effects obtained the streaming percentage (fraction of all cells that show streaming) is estimated.

All cations, which were administered as chlorides with the exception of aluminium, show an influence specific for the ion. Many cations will be adsorbed even at the low salt concentrations used, owing to the preponderantly negative charge of the plasm. This may be one

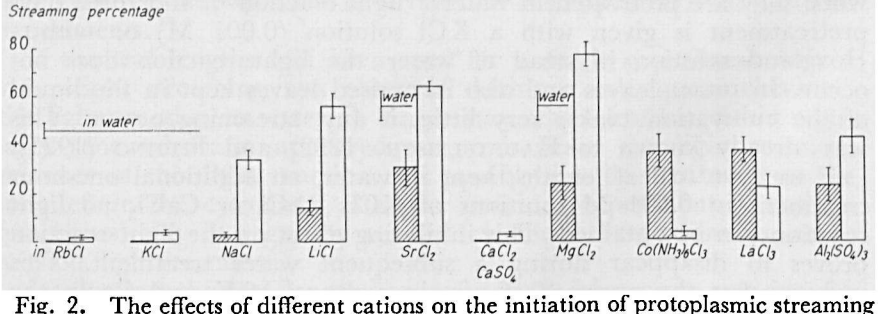

Fig. 2. The effects of different cations on the initiation of protoplasmic streaming in subepidermal cells of *Vallisneria* leaves. The effects are determined in the dark, ½–1 hour after addition of the salt solution (black blocks) and after exposure (open blocks). See explanation of fig. 1.

reason why the cation effects are more pronounced than the anion effects.

The inhibition of the light reaction by cations progresses according to the subjoined series: (fig. 2)

$$\begin{array}{lll} \text{Rb} \geqslant \text{K} > & \text{Na} > (\text{H}_2\text{O}) & \text{Li} \\ \quad \text{Ca} \lll & & \text{Sr} > \text{Mg} \\ & \text{Al} & \\ \text{strong inhibition} & \text{decreasing inhibition} & \end{array}$$

Initiation of the protoplasmic streaming in the dark takes place according to the following series:

$$\begin{array}{lll} \text{Rb, K} < & \text{Na} < \text{Li} & \\ \quad \text{Ca} \lll & \text{Mg, Sr} & \\ & \text{Al} < \quad \text{La, Co}(\text{NH}_3)_6 & \\ \text{no stimulation} & \text{increasing stimulation} & \end{array}$$

On our comparing the two series, we see that the cations that do not inhibit the light reaction to an appreciable extent do initiate streaming in the dark. So the streaming percentages found after exposure are for these cations only partly the result of the action of the light. It is a striking fact that the ions normally occurring in the plasm, i.e. Ca and K strongly inhibit the light reaction (the Rb behaves like K). The other cations give a slight inhibition, if any. $Co(NH_3)_6$ and La are injurious to such a degree that after an hour's action no light reaction is found.

The above series show similarity with the lyotropic series as given by KAHO (1926) and more recently by FISCHER (1956). As compared to the permeability series the Ca takes an exceptional place in our series.

An antagonism between K and Ca was not noticeable in our experiments. Therefore permeability phenomena are of minor importance in these experiments.

From the course of the series is to be seen that other properties of the cations than charge and hydration are more important for the phenomena occurred, although the hydration seems to play a role of more importance in the effects brought about by the cations of the monovalent series. The hydration of di- and trivalent cations also may be of importance, but is not observed because effects of other properties of these cations predominate. The effects we get, however, are not only dependent on the properties of the ions but also on the properties of the groups of the plasm the ions are acting upon. Next to this the state of protoplasm i.e. quiescent or streaming protoplasm is of importance.

It should be observed that the inhibitory effect of KCl on the initiation of protoplasmic streaming by light is only found, if this salt has previously acted on quiescent protoplasts. If there is already some protoplasmic streaming when the KCl is supplied, this streaming continues and no inhibition of this streaming occurs. So K does not

inhibit the process of streaming as such, but prevents the initiation of streaming by exposure to light or by asparagine.

Different causes can initiate protoplasmic streaming (SEIFRIZ 1943). We can imagine that their ultimate effect will be a giving rise to changes in the protein molecules of the plasm. In consequence of this, changes may occur in the protoplasmic structure, which may lead to changes in plasmic viscosity. Changes in plasmic viscosity have according to Seifriz an influence on the rate of protoplasmic streaming. VIRGIN (1949) found that the rate of streaming is increased if the plasmic viscosity is decreased. SCHAEFER (1958) thinks a viscosity decrease of importance for the initiation of protoplasmic streaming. An influence of the moving force of the streaming is also important.

From the literature facts are known on the influence of light on the protoplasmic streaming. Red light gives the strongest acceleration of the rate of protoplasmic streaming in cells of *Vallisneria* and *Elodea* (BEIKIRCH 1925) and has also the greatest effect on the initiation of protoplasmic streaming in mesophyll cells of *Vallisneria* (SCHWEICKERDT 1928); the effect of blue light is considerably slighter. According to VIRGIN (1951, 1952, 1954) blue light has the greatest effect on alterations in the plasm viscosity in leaves of *Elodea* densa, red light having a slight influence. Dependent on the light intensity protoplasm of *Elodea densa* leaves shows an increase or a decrease in viscosity (VIRGIN 1951). The transition dark-light always initiates the protoplasmic streaming in mesophyll cells of *Vallisneria* in this range of light intensities (FITTING 1925, SCHWEICKERDT 1928). The length of the exposure period is important for obtaining a certain effect. The above indicates that the initiation of protoplasmic streaming by light does not take place under the influence of a decrease in plasmic viscosity. Both processes are induced by light via different mechanisms. Probably chlorophyll plays a part in the initiation of protoplasmic streaming by light in objects containing chlorophyll.

From some of our experiments in which the plasm viscosity is determined, something similar appears. The viscosity (determined from the degree in which the plasm and its inclusions are moved by centrifugal force) of the plasm of *Elodea densa* leaves and *Vallisneria* leaf lengths proved to be increased after a ten minutes' exposure to light (intensity about 550 M.C.). The viscosity of the plasm of leaves or leaf lengths, treated with 0.001 M solutions of KCl, KCNS or $MgCl_2$ during one hour is increased as compared with leaves in water. Under the influence of the last two salts streaming is initiated. The difference found in viscosity of the plasm in the leaf cells treated with the different salts is not significant. These results, especially the effect of exposure, indicate that a decrease in plasmic viscosity will be of little importance for the initiation of protoplasmic streaming.

Changes in energy supply may have an influence on the rate of protoplasmic streaming. The effect of sugar already points in this direction.

According to GOLDACRE (1952) and FREY-WYSSLING (1953, 1955) the protoplasm should have the following properties which are

conditions for the occurrence of protoplasmic streaming: 1° the possession of contractile proteins and 2° a reversible gel-sol transformation.

For the contraction of these proteins energy is needed, originating from ATP (SZENT GYÖRGY 1947, GOLDACRE and LORCH 1950, KAMIYA, NAKAYIMA and ABE 1957). In the object with which the latter investigators worked (*Physarum polycephalum*) LOEWY (1952) demonstrated contractile actomyosine like proteins.

According to GOLDACRE (1952) the plasm gel consists of predominantly stretched chains of proteins which pass by contraction into folded units forming the sol and becoming again part of the plasm-gel by unfolding later. The stretched chains have a great adsorbing power for instance for cations, because polar (and other) groups of the secondary chains have been set free for the most part. The folded chains possess this power in a much less degree, owing to these groups being bound together.

It may be imagined that salts may influence the system of the plasmic streaming as regards the supply of energy and the reversible gel sol transformation. According to BOGEN (1951) small changes in charge of the protein molecules would exert an influence on the contraction and relaxation of these. Changes in charge of the protein molecules as brought about by the adsorption of cations may be a cause of an initiation of contraction. It is, however, clear from our experiments that beside charge other properties of the cations are of importance.

Cations may influence enzyme systems which are used for metabolism and exchange of energy. It is for instance known that Mg activates glycolysis (MC ELROY and NASON 1954) which may lead to an increased ATP production (HAAS 1955). This may effect protoplasmic streaming.

Apart from this specific influence an aspecific influence exists. In this case cations may influence the enzyme properties by changing charge and hydration of the carrier protein involved.

The effect of light on the induction of protoplasmic streaming might be based on a formation of ATP by the chlorophyll system (ARNON 1956).

Another possibility is that the cations act upon the ATP-ases which are involved in the contraction of the protein chains directly. The contractile proteins themselves possibly have ATP-ase properties owing to their being entirely or partly ATP-ase as DANIELLI (1952) conceives it and as was found by ENGELHARDT and LJUBIMOVA for the actomyosine complex. Modifications in the cation composition both qualitative and quantitative greatly affect the properties of actomyosine. It may be imagined that similar modifications occur under the influence of salts in the contractile proteins of the *Vallisneria* plasm and that the phenomena found are partly due to these.

Trivalent cations will be capable of causing gel-formation through their strong adsorption. This may explain why under the influence of $Co(NH_3)_6$ and La the streaming percentage decreases with the

time. Aluminium does not show this phenomenon in our experiments. Szücs (1910) found, however, that the plasm of *Vallisneria* under the influence of low Al concentrations was consolidated to such a degree that it was not moved by centrifugal force. This indicates a gel formation.

It has appeared that K, Rb and Ca inhibit the light reaction. Mg does not inhibit the light reaction, but rather stimulates it. In a Hoagland solution the influence of the Mg is hardly or not at all perceptible. The effect of Ca and (or) K is therefore antagonistic with respect to Mg. It may be that owing to the very lack of K or Ca or both, certain cations can exercise their typical influence on the plasm.

For the transport from cell to cell of K, Rb and Ca and of anions given in the medium besides these cations, the protoplasmic streaming in this object will be immaterial (See Arisz 1956, p. 49).

SUMMARY

Through exposure protoplasmic streaming appears in subepidermal cells of leaf lengths of *Vallisneria* pretreated with water (light reaction). The light reaction is not forthcoming when before exposure the leaf lengths have been treated for an hour with 0.001 M concentrations of KCl, KNO_3, $KHCO_3$, K_2SO_4, KH_2PO_4, $CaSO_4$ and RbCl. The inhibition of the light reaction under the influence of these salts is reversible. By Na, Li, Mg, Sr, La and $Co(NH_3)_6$-chloride, Al sulphate and KCNS streaming is already induced in the dark in different degrees; the light reaction is not inhibited or only little. Under the influence of $Co(NH_3)_6$ and La the streaming percentage decreases with the time; a light reaction does not occur: these salts cause damage. The effects of salts are mainly cation effects. The inhibition of the light reaction by KCl affects the initiation of the streaming, not the streaming as such.

Modifications in the viscosity of the plasm, as determined from experiments with centrifugal force are probably immaterial for the initiation of protoplasmic streaming. It has been assumed that salts modify the properties of contractile proteins directly or influence the energetic system of protoplasmic streaming too.

ACKNOWLEDGEMENT

This investigation was performed at the instigation of Prof. Dr. W. H. Arisz. I am much indebted to him for his advice and his valuable criticism.

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