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THE SECRETION OF THE SALT GLANDS OF LIMONIUM LATIFOLIUM KTZE

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INTRODUCTION

In a great number of plants living in saline habitats salt glands are found in the leaves. Among them are the Plumbaginaceae, the Frankeniaceae and some genera of the Gramineae and Tamaricaceae, as *Spartina*, *Aeluropus* and Tamarix. FABER (1923) demonstrated salt glands in Mangroves (*Avicennia marina*).

SCHTSCHERBACK (1910) studied the secretion in excised leaves of *Statice*. Structure and function of the salt glands of *Statice Gmelini* were fully investigated by RUHLAND (1915). Skelding and Winterbotham described the salt glands in the leaves of *Spartina Townsendii*.

Ruhland showed that the glands of *Statice Gmelini* consist of a complex of 16 cells rich in cytoplasm and with large nuclei (Fig. 1). The diameter of the glands is 43–46 μ. There were found on the upperside of the leaf 722 glands and on the underside 644 glands per sq. cm. Vacuoles either do not occur in the gland cells or are but of a small size.

On the outside the complex of gland cells is covered by a cutinized membrane with a cuticle, in which there are four pores (P), one in each secreting cell (A). At these places the fluid emerges. The pressure in these cells cannot extrude the cytoplasm through these pores, since a kind of membrane is present, which closes the opening in the cell wall. Between the gland cells and the adjoining cells of the tissue there is a cutinized wall which encloses the gland on all sides. Four collecting cells are bounding the gland on the side of the mesophyll. Communication between mesophyll and the cytoplasm of the outer gland cells (the cupcells) is only possible by means of these collecting cells. These cells are provided with some passage spots lying in the cutinized walls bounding the gland.

Ruhland corroborated Schtscherback's observation that leaf-segments and also isolated glands on water are capable of secretion. He arrived at the conclusion that under fairly high pressure the glands give off a fluid of which the sodiumchloride concentration is equal to or somewhat higher than the NaCl concentration in the leaf cells.

An increase of the chloride concentration in the gland he could not prove. He determined the osmotic value of the secreted salt solution by the Barger method. The difference between the osmotic concentration of the sap of the gland and the NaCl content he sometimes found to be fairly large. This must be due to the presence of organic substances.

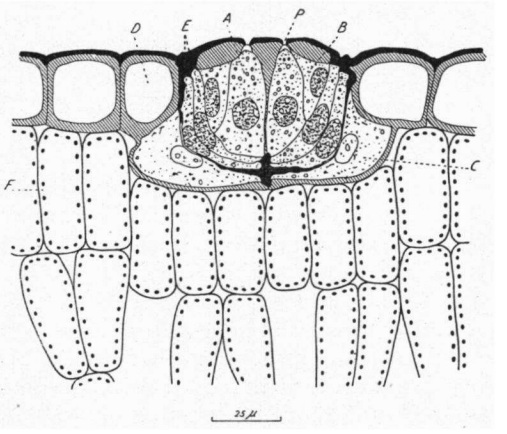

Fig. 1. Gland of *Statice Gmelini* according to Ruhland, slightly altered.
A. secretion cell; P. Pore; B. adjoining cell; C. collecting cell; D. epidermal cell; E. cup cells; F. mesophyll.

After Ruhland's publication the salt glands have not been fully examined any more. They do, however, deserve attention, being organs of extraordinary efficiency in removing salt from the tissue. Ruhland found a secretion of liquid of 0.287–0.861 mg per hour per sq.cm. Our values for *Limonium latifolium* correspond with this. A 150 mg. leaflength secretes 70 mg in 24 hours.

Ruhland's fundamental experiments have been repeated and extended in my laboratory by some younger students, each working during a short period, Miss I. J. Camphuis in 1946, Miss H. Heikens in 1950 and Mr A. J. van Tooren in 1954. The results obtained have been summarized here together with some observations of my own. The plant used for these researches was *Limonium latifolium* Ktze (*Statice Limonium pseudolimonium*). In autumn potplants kept in a hot house were also used, which still showed secretion in that season.

Method

If a leaf or leafsegment secretes a liquid, the drops remain on the leaf and it will therefore depend on the vapour tension of the air whether evaporation of water from the drops or condensation of watervapour from the air takes place on the leaf. In order to prevent a change in concentration of the secreted fluid glass rings of 21 mm inside diameter and 3 mm height were fixed with Leicks'wax on leaf

disks 3 cm in diameter, punched from the leaf. A closed chamber was obtained by fixing a glass plate on the ring with vaseline. In this way changes in concentration of the secreted drops could occur only in a very slight degree. Leafdisks 3 cm in diameter were punched from a leaf on either side of the midrib. The behaviour of disks lying on the same level was compared. The disks were put on filter paper in a dish containing water or salt solution.

After the experiment the secreted fluid in the chambers was sucked up into filter paper that had been previously dried and weighed, and the total weight was determined in a weighing bottle. The chloride content was determined after destruction of the filterpaper with HNO_3 in which $AgNO_3$ had been solved. Chloride was determined by the Volhard method.

The determination of the osmotic value of secreted fluid and sap expressed from the leaves was executed by Baldes and Johnson's osmometric method worked out in this laboratory by VAN ANDEL (1952). As comparative fluid a NaCl solution was used. The expressed sap was obtained after killing and expressing the leafdisks in a hydraulic hand press at a definite pressure. The secretion shows a great variability. By our choosing suitable leaves and gathering the results of various leaves in one experiment figures could be obtained rendering an orientation about the influence of various factors on the secreting process possible.

PRETREATMENT

Leaves of a plant from the garden contain but little chloride, about 0.23 to 0.37 %. They show no secretion. In order to obtain a proper salt secretion the intact leaves are placed beforehand with their petioles into a salt solution. Next disks 3 cm in diameter are punched from the leaves. In disks from leaves which have been for 3 hours with their petioles in 0.25 M NaCl, and which have next been placed on water, the secreted fluid amounted to 40 μg Cl in 20 hours, after a 6 hours' pretreatment 127 μg and after 22 hours 265 μg per 20 hours. The leafdisks take up the NaCl through the edges of the cut. If the latter have been greased with vaseline, no more salt is taken up.

In orientating experiments it appeared that according as the NaCl concentration is higher in the pretreatment, the chloride concentration of the secreted fluid increases. If the secretion of the leafdisks takes place on water, the increase is considerably smaller than if it takes place on a NaCl solution. In the first case no salt can be taken up during the secretion, so that owing to the loss of salt the concentration in the leaf is reduced, in the second case the salt absorption is continued, which can now be stronger, equal to or weaker than the salt secretion. This will depend on the NaCl concentration of the external solution and on the activity of the secretion process. So we found with pretreatment and secretion of the leaf on 0.08 M NaCl solution a NaCl concentration of the secreted fluid of 0.31 M; this rises on a higher concentration and amounts to 0.72 M on 0.51 M NaCl. When the

secretion took place while the leaf was on water, the concentration of the secreted fluid amounted to 0.16 and 0.23 M respectively.

The use of chambers to prevent a change in concentration of the secreted fluid proved to be important. Without chambers too low concentrations of the secreted fluid were found on external solutions of low concentration, because water from the air condensed on the drops. On solutions of high concentration (over 0.42 M) too high concentrations were found without chambers, because in this case water evaporated from the drops.

TABLE I

Influence of pretreatment (24 hours) on the secretion at 25° C during 24 hours. 1951.

NaCl concentration during pretreatment	quantity secreted in mg	Cl in μg	M Cl conc. secreted fluid	M Cl conc. in leaf
Secretion on 0.47 M NaCl				
0.085 M NaCl (0.5 %) . .	53	931	0.49	0.17
0.171 „ „ (1.—) . .	31	381	0.34	0.18
0.342 „ „ (2.—) . .	40	656	0.46	0.28
0.513 „ „ (3.—) . .	31	596	0.54	0.39
0.684 „ „ (4.—) . .	13	171	0.35	0.16
Secretion on water				
0.085 M NaCl	48	134	0.08	0.08
0.171 „ „	41	124	0.08	0.11
0.342 „ „	60	299	0.14	0.14
0.513 „ „	42	329	0.22	0.22
0.684 „ „	37	278	0.21	0.31

Table I gives the influence of a pretreatment on various concentrations NaCl from 0.5 to 4.0 %, secretion taking place either on 2.75 % (0.47 M) NaCl or on water. A pretreatment on 2 % (0.34 M) NaCl is optimal. Both the chloride concentration of the secreted fluid and that of the leaf have been determined. When the secretion takes place on water these quantities are fairly equal. When secretion takes place on 0.47 M NaCl the concentration both in the leaf and in the secreted fluid is greatest after pretreatment on 3 % (0.51 M) NaCl. So there appears to be a distinct after effect of the amount of salt taken up during the pretreatment. In the subsequent experiments the pretreatment has been standardized as much as possible. In the experiments in 1950 pretreatment took place for 24 hours in the dark at 25° C on an external solution of 0.47 M NaCl. In 1954 pretreatment took place in artificial light at 20° or 25° on 0.34 M NaCl.

The time course of the secretion process

It has already been discussed that a leafdisk placed on water after having taken up an amount of NaCl during the pretreatment, secretes so much chloride at 25° C that the Cl concentration in the leaf is

considerably reduced (Table II). This is accompanied by a fall in Cl concentration in the secreted fluid.

TABLE II

Changes in Cl concentration during secretion in leaf and secreted fluid. 1946. Pretreatment on 0.42 M NaCl. Secretion on water.

	M Cl conc. in leaf	M Cl conc. in secreted fluid
At the beginning	0.06	
24 hours pretreated.	0.25	
22 hours secretion on water	0.17	0.15
44 hours secretion on water	0.16	0.06
66 hours secretion on water	0.13	0.06

TABLE III

The time course of secretion on a salt solution and on water. 1950. Pretreatment 24 hours, dark, 25° C on 0.47 M NaCl solution.

	Secretion on 0.47 M NaCl (4 discs)			Secretion on water (6 discs)		
	quantity in mg	Cl μg	M Cl conc.	quantity in mg	Cl μg	M Cl conc.
first day	22	517	0.66	28	324	0.32
second day	25	508	0.58	31	196	0.18
third day.	22	410	0.53	32	76	0.06

Cl conc. at the beginning in leaf 0.59 M
at the end on Na Cl . . 0.39 M
at the end on water . . 0.14 M

Table III gives the course of the secretion during 3 days. During the secretion part of the leaves were placed on 0.47 mol NaCl, another part on water. When secretion took place on the salt solution, the Cl concentration of the leafdisks decreased from 0.59 to 0.39 M. The secreted fluid had a higher Cl concentration than the average Cl concentration of the leaf amounted to. The amount of secreted fluid remained fairly equal in the successive days. The Cl content of the leafdisks on water was much more reduced, from 0.59 to 0.14 M. Also in this case the amount of secreted fluid remained fairly equal on the 2nd and the 3rd day and was slightly higher than in the experiment with leafdisks on salt solution. The concentration of the secreted fluid was greatly reduced, from 0.32 to 0.06 M NaCl. It seems that at the end of this experiment a slightly higher salt concentration was present in the leaf than in the secreted fluid. If this difference is significant, it indicates that not all chloride present in the leaf can be given off by secretion. Such a regular rate of secretion during three days, which is besides nearly the same on salt solution as on water, was not found in most experiments. Usually the rate of secretion is already reduced on the second day. In Tables IV and V the secretion has been examined during 2 or 3 days for leaf disks on 0.47 M NaCl and on water. The amounts of fluid exuded on salt solution and on

water are fairly equal, but both on salt solution and on water they are smaller on the second and the third day. This reduction of the secretion on the second and the third day is independent of the salt concentration of the secreted fluid, the NaCl concentration of the secreted fluids being different on salt and on water. This indicates that

TABLE IV

The time course of secretion on a salt solution and on water. 1950. Pretreatment as in Table III.

	Secretion on 0,47 M NaCl (4 discs)			Secretion on water (5 discs)		
	quantity in mg	Cl μg	MCl conc.	quantity in mg	Cl μg	MCl conc.
first day	66	1444	0.62	67	652	0.27
second day	48	950	0.56	42	183	0.12

the amount of secreted fluid is independent of the salt concentration of the gland sap. In the course of nearly all experiments the exuded amount of fluid is reduced in successive periods. It should be borne in mind that the experiments took place in the dark. It is therefore interesting to know whether on exposure to light the strength of the secretion has a course different from that in the dark.

TABLE V

The time course of secretion on a salt solution and on water. 1946. Pretreatment 22 hours, dark, 0.42 M NaCl solution.

	Secretion on 0.42 M NaCl			Secretion on water		
	quantity in mg	Cl μg	M Cl conc.	quantity in mg	Cl μg	M Cl conc.
first 22 hours . . .	67	929	0.39	71	383	0.15
second 22 hours . .	38	542	0.42	37	198	0.15
third 22 hours. . .	20	336	0.47	16	29	0.05

The influence of light on the secretion appears from a comparative experiment in which after the usual pretreatment half of the leafdisks are exposed to the light, the other half put in the dark on 2 % NaCl (0.342 M). The average amount of secreted fluid in the light per disk is 50 mg with 0.544 M Cl, in the dark 16 mg with 0.520 M Cl.

TABLE VI

Influence of exposure to light on secretion. 1954. Pretreatment 24 hours, 25° C 0.342 M NaCl solution.

Secretion 24 hours 25° C on 0.342 M NaCl			
in	amount in mg	M NaCl conc.	osmometric det.
light.	97.6 (4)	0.384	0.379 M NaCl
dark.	64.1 (4)	0.393	0.413 M NaCl

In a second experiment a slightly smaller difference was found between light and dark (Table VI). During the first 24 hours 97.6 mg

was secreted in the light with 0.384 M NaCl and in the dark 64.1 mg with 0.393 M NaCl. In the second 24 hours the secretion was greatly reduced both in the light and in the dark, 34.75 and 28.9 mg. In this case the osmotic value of the secreted fluid was determined with the aid of an osmometer. The concentration of the sap in the osmometer being compared with a known concentration NaCl, the osmotic values obtained have been expressed in the isotonic NaCl concentration. In the light the osmotic value was 0.379 M NaCl. In the dark 0.413 M NaCl. These values do not quite correspond with the osmotic value which may be computed from the Cl determinations. The differences, however, are too small to give an indication whether osmotically working substances other than NaCl occur in the secreted fluid.

From these experiments we may draw the conclusion that the rate of secretion is promoted by exposure to light.

Influence of the temperature on the secretion

The influence of the temperature on the secretion process was investigated at 5° C and at 25° C (Table VII). Experiment A shows the secretion at 5° and at 25° C of leafdisks, one half of which secreted on water, the other half on 2.75 % (0.47 M) NaCl solution. In B and C secretion takes place at 5° C on the first day, at 25° C on the second day. From the tables it appears that when secreted on 0.47 M NaCl solution the amount of secreted fluid at 25° C is much greater than at 5° C, but that the NaCl concentration of the secreted fluid is equal at the two temperatures.

TABLE VII

Influence of temperature on secretion. Pretreatment 24 hours, dark, 25° C, on 0. 47 M NaCl solution.

A 1950 24 hours	Secretion on 0.47 M NaCl			Secretion on water		
temp.	amount in mg	Cl μg	M Cl conc.	amount in mg	Cl μg	M Cl conc.
5° C	6.6 (2)	132	0.56	13.6 (2)	268	0.56
25° C	58.0 (3)	1188	0.58	51.0 (3)	399	0.22
B 1950						
first day 5° C . .	6.4	141	0.62	10.6	220	0.60
sec. day 25° C . .	43.6	951	0.62	76.6	629	0.23
Cl content at the beginning in leaf 0.47 M after two days on NaCl 0.42 M after two days on water 0.20 M						
C 1951						
first day 5° C . .	14.0	297	0.60	22.0	320	0.41
sec. day 25° C . .	59.0	1242	0.59	78.0	527	0.19

The salt concentration in the leaf is but slightly reduced, when secretion takes place on a salt solution. If secretion takes place on water, the concentration in the leaf after the second day has got

considerably reduced. At 5° C the amount of fluid secreted on water is greater than on 0.47 M NaCl solution. This must be due to the lower osmotic value of the medium and the resulting stronger turgor in the gland cells. The concentration of the secreted fluid flowing out at 25° C on water is considerably lower, because at this temperature much more sap containing NaCl is given off and as a result the Cl concentration in the leafdisk is considerably reduced, in B from 0.47 to 0.20 mol NaCl.

The temperature therefore has no influence on the concentration of the exuding sap and only regulates the amount of sap secreted.

Dependence of the secretion on oxygen

The influence of withdrawal of oxygen on the secretion was investigated in some experiments. Decrease in oxygen content of the air was obtained by binding the oxygen by means of hydrogen in a McIntosh and Fildes anaerobic jar.

The oxygen was bound to the added hydrogen gas by means of a palladium catalysator, the jar being free from oxygen after about half an hour. The withdrawal of the oxygen was checked by means of reduced aqueous methylene blue. After a 24 hours'pretreatment with 0.47 M NaCl solution the leaf disks were put in the jar for 24 hours on 0.47 M NaCl solution. Leafdisks in aerobic conditions gave 29 mg of secreted fluid with 570 μg Cl and a NaCl concentration in the secreted fluid of 0.39 M. In the absence of oxygen the secretion in 5 leafdisks averaged 1.1 mg. The secreted fluid did not contain any demonstrable Cl. Next the leafdisks were put in ordinary air, but they were no more capable of secreting.

Influence of inhibitors on the secretion

The influence of some substances inhibiting metabolism was investigated in 1950. They were KCN, Naazide, Iodoacetic acid (I.A.A.), arsenite, NaF, 2,4-dinitrophenol and phloridzine. At a low concentration KCN, I.A.A. and NaF give a distinct stimulation, arsenite a somewhat weaker one, whereas azide does not give a distinct stimulation. At a higher concentration they all give an inhibition of the secretion. Phloridzine had no influence. The results with dinitrophenol were variable (Table VIII).

TABLE VIII

Influence of inhibitors on secretion process. 1950. Pretreatment 24 hours dark 25° C on 0.47 M NaCl.

	Secretion 24 hours 25° C on 0.47 M NaCl solution	
	conc. of inhibitor giving stimulation	conc. of inhibitor giving 50 % inhibition
KCN	0.25×10^{-3} M	$7\text{–}8 \times 10^{-3}$ M
I.A.A.	5×10^{-5} M	$2\text{–}4 \times 10^{-3}$ M
Naazide		$2.5\text{–}7.5 \times 10^{-3}$ M
NaF	$7.5\text{–}25 \times 10^{-4}$ M	7.5×10^{-3} M
arsenite	10^{-7} — 10^{-6} M	$10^{-3}\text{–}5 \times 10^{-3}$ M
2,4-dinitrophenol	?	5×10^{-4} M

On determining the correlation between the amount of secreted fluid and the amount of Cl given off in a certain time with various concentrations of KCN and relating these values to the amounts of secreted fluid and Cl given off by the disks without KCN, we see that the correlation between the alterations in the two quantities under the influence of inhibitors is very great. For KCN it amounted to + 0.96 for I.A.A. + 0.91 and for Na-arsenite + 0.96. From this it follows that the concentration of the secreted fluid under influence of the inhibitors does not alter significantly. The inhibitors therefore only affect the rate of secretion.

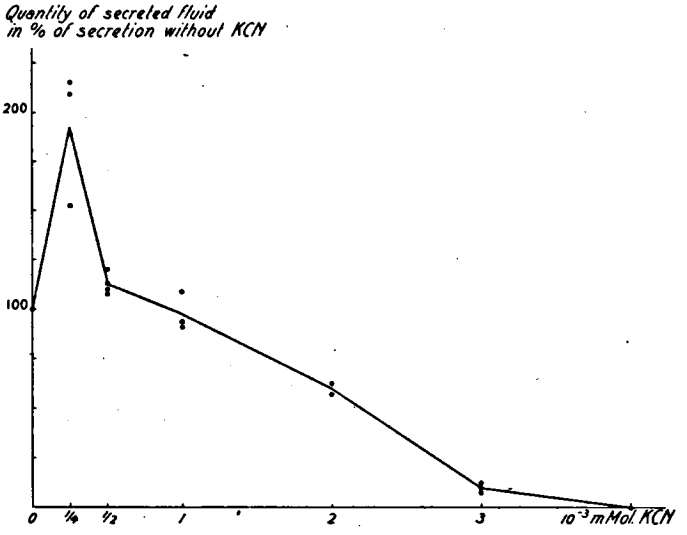

Fig. 2. Influence of KCN on the amount of liquid secreted by the salt glands of *Limonium latifolium;* the concentration remains unaltered.

The influence of KCN on the amount of secreted fluid expressed in percentage of the control without KCN is shown by Fig. 2. There is a distinct stimulation at 0.25 and 0.5 $\times$ 10^{-3} M KCN, at a higher concentration the secretion is reduced to 0. Table IX shows the influence of various KCN concentrations on the rate of secretion expressed in percentages of the control experiment without KCN.

TABLE IX

Influence of KCN on secretion. 1954. Pretreatment 24 hours, 20° C, 0.342 M NaCl, exposed to light.

Secretion 24 hours, 20° C, dark, on 0.342 M NaCl Quantity of secreted fluid expressed in percentage of control without KCN						
conc. KCN	0.00025	0.0005	0.001	0.002	0.003	0.004 M
	215	110	90	62	10	0
	152	120	94	57	8	0
	209	108	109		12	
	189	113				
Average value	191	113	98	60	10	0

Relation between the osmotic values of the external solution, of the expressed sap of the leaf and of the secreted fluid

From the data of Tables I, II, III and VII it appears that leaves that secrete on water have a chloride concentration in the secreted fluid which is equal to or lower than that of the leaf. Leaves that secrete on a salt solution have a higher chloride concentration in the secreted fluid than in the leaf. Table X shows the same phenomenon. The chloride concentration of the leaf being a quantity computed from the fresh weight of the leaf and the chloride analytically demonstrable does not imply that in the cytoplasm of the leaf cells this chloride concentration really exists. To get an impression about this, it would be necessary to know whether the chloride concentration in cell wall, cytoplasm and vacuole was equally high. A comparison of the Cl concentration in the leaf and in the secreted fluid therefore has only a limited value and no conclusion should be drawn from it concerning a possible salt accumulation in the glands.

TABLE X

Relation between the concentration of NaCl in leaf and in secreted fluid. 1946. Pretreatment 24 hours, dark, 0.42 M NaCl.

	Secretion on 0.42 M NaCl		Secretion on water	
M Cl conc.	in leaf	in secreted fluid	in leaf	in secreted fluid
at the beginning	0.06		0.06	
after the pretreatment . .	0.25		0.25	
after 22 hours.	0.30	0.39	0.17	0.15
after 44 hours.	0.28	0.45	0.16	0.06
after 66 hours.	0.23	0.33	0.13	0.06

An osmometric determination of the secreted fluid can be made with a good result with the aid of the osmometer (Van Andel, 1952). In table VI it has already been found that the osmotic value found for the secreted fluid (secretion on 0.34 M NaCl solution at 25° C) expressed as an isotonic NaCl solution, corresponds with the NaCl concentration of the secreted fluid analytically determined. Both in the dark and in the light this value lies considerably higher than the osmotic value of the external solution.

It was thought desirable to compare the osmotic value of the secreted fluid also with that of the expressed leaf sap (Table XI). For the osmotic value of the sap of the leaf rather divergent values were found, but the osmotic value of the secreted fluid was always higher than that of the leaf sap. In this case too there was a great correspondence between the osmometrically determined value of the secreted fluid and the analytically found one. The differences lie within the limits of error.

These determinations had been made with leaves lying on a 2 % NaCl solution (0.342 M). In this experiment the NaCl concentration of the secreted fluid was higher than the NaCl concentration of the

medium. The average osmotic concentration of the leaf sap corresponds with that of the external solution. In some experiments the osmotic concentration is higher, in the others lower than that of the external solution. This will depend on the amount of other substances than NaCl present in the sap.

TABLE XI

Osmotic conc. of leaf sap and secreted fluid. 1954.

Secretion 24 hours, 25° C, on a 0.342 M NaCl solution.		
Leaf sap osmometric determination	secreted fluid osmometric determination	secreted fluid NaCl conc.
0.406 M NaCl	0.463 M NaCl	0.451 M
0.411	0.461	0.454
0.346	0.402	0.408
0.275	0.425	0.414
0.305		0.409
0.292		0.415
0.319		0.394
0.348		0.411
Av. 0.338		0.420

The osmotic value of the secreted fluid being higher than that of the leaf sap while this is about equal to that of the external solution, it is evident that the glands are capable of doing osmotic work.

TABLE XII

Influence on the secretion of increasing the osmotic value of the outer solution by adding sugar. 1954.
Pretreatment 24 hours, 25° C, 0.342 M NaCl, exposed to light.

Secretion 2 periods of 24 hours, 25° C, dark			
First period. secretion	quantity in mg	Cl μg	conc. secreted fluid
0.342 M NaCl	37.2	771	0.58 M
„ „ „ + 0.071 M sucrose	24.8	593	0.67
„ „ „ + 0.142 „ „	21.8	655	0.85
Second period.			
0.342 M NaCl	22.2	457	0.58
„ „ „ + 0.071 M sucrose	16.9	380	0.63
„ „ „ + 0.142 „ „	11.9	398	0.97

To investigate the influence of the concentration of the external solution on the secretion process sucrose of various concentrations has been added to the outer solution (Table XII). Under the influence of the sucrose added the amount of secreted fluid is reduced and its concentration increases. So the influence of the sucrose addition is clearly shown. It may be due to the osmotic increase of the external solution or to the supply of sugar as a source of energy or to both influences. In Table XIII the influence of the increase of osmotic value of the outer solution through addition of higher sugar concentrations on the NaCl concentration of leaf and secreted fluid has been traced.

As already discussed above the NaCl concentration in the secreted fluid is considerably higher than in the leaf in such cases. Addition of sugar gives a lower rate of secretion. The NaCl concentration in the leaf remains about the same, that in the secreted fluid is considerably increased with the greatest addition of sugar.

TABLE XIII

Influence of increasing the osmotic value of the outer solution on the NaCl conc. in leaf and in secreted fluid. 1954.
Pretreatment 24 hours, 25° C, 0.342 M NaCl, exposed to light.

Secretion 24 hours, 25° C, dark			
	quantity in mg	M Cl conc. in secreted fluid	M Cl conc. in leaf
Secretion on 0.342 M NaCl	31.9	0.418	0.336
„ „ „ „ „ + 0.150 M sucrose	15.4	0.378	0.318
„ „ „ „ „ + 0.250 „ „	11.8	0.501	0.324

TABLE XIV

Influence on the secretion of increasing the osmotic value of the outer solution by adding sugar. 1954.
Pretreatment 28 hours, 25° C, 0.342 M NaCl, exposed to light.

Secretion 24 hours, 25° C, dark			
	quantity in mg	M Cl conc.	osmotic value osmometrically determined
Secretion on 0.342 M NaCl	38.2	0.358	0.412
„ „ „ „ „ + 0.086 M sucrose	37.0	0.558	0.613
„ „ „ „ „ „ + 0.172 „	17.6	0.688	0.750

Table XIV shows the influence of an increase in osmotic value of the external solution through addition of sugar on the osmotic value of the secreted fluid. In this experiment both an analytical determination of the NaCl concentration of the secreted fluid and an osmometric determination of the NaCl concentration which is isotonic with the secreted fluid was made. It appears that in every case the osmometrically determined value lies higher than the NaCl concentration of the secreted fluid, so that besides NaCl a small amount of a different substance must have been present in the secreted fluid. Through addition of sugar here too the NaCl concentration of the secreted fluid increases greatly.

In Table XV experiments are shown in which $MgSO_4$ has been added to the external solution instead of sugar, in order to investigate a substance which would act especially osmotically. NaCl has been applied in two different concentrations as well.

Here too an addition of NaCl brings about a somewhat smaller amount and a higher concentration of the secreted fluid, resulting in a higher amount of Cl given off. On enhancing the concentration with $MgSO_4$ a considerably smaller amount of secreted fluid is obtained,

while the Cl concentration of the secreted fluid increases less. The result is a smaller total amount of Cl being given off than without the addition of $MgSO_4$. In principle the influence of sugar, NaCl and $MgSO_4$ corresponds, so that the action of these substances is based in the first place on their osmotic influence.

TABLE XV

Influence on the secretion of increasing the osmotic value with magnesium sulfate. 1954.
Pretreatment 28 hours, 20° C, 0.342 M NaCl, exposed to light.

Secretion 28 hours, 20° C, dark	quantity in mg	Cl μg	M conc. NaCl in secreted fluid
secretion on 0.171 M NaCl	50.3	516	0.288
„ „ „ „ „ + 0.081 M $MgSO_4$	25.0	326	0.366
„ „ 0.342 M NaCl	42.4	647	0.429

MECHANISM OF THE GLAND SECRETION

The action of the gland cells is considered by Ruhland as an active process secreting water in which salts and organic substances are present in a varying quantity. If the gland cell is rich in salt, the secreted fluid is rich in salt as well, if the gland cell is poor in salt, if for instance the secretion takes place on water, the secreted fluid is also poor in salt. In his opinion the glands work without selectivity. They are no filtration hydathodes, but they squeeze out water actively. Osmotic work is not turned out by the gland cells according to Ruhland.

Our data are not yet adequate to give a full explanation of the secreting process. They supplement Ruhland's results and differ from them mainly in one respect, as we found that the osmotic value of the secreted sap can be higher than that of the expressed sap of the leaf, i.e. that the glands can perform osmotic work. It was proved that secretion is an active process dependent on the presence of oxygen and demonstrated that temperature, light and inhibitors influence secretion. They change the rate, but leave the NaCl concentration unaltered. This proves that the secretion process depends on cell metabolism.

In a leaf on a salt solution the NaCl concentration of the secreted fluid is higher than the NaCl concentration of the leaf. It being unknown, whether the chloride present in the leaf is found in wall, cytoplasm or vacuole, nothing can be stated about the actual NaCl concentration in the cytoplasm of the leaf cells and it is therefore unknown whether the NaCl concentration in the secreted fluid is actually higher than in the cytoplasm of the leaf cells.

The osmotic value of the expressed sap of the leaf seems to correspond more or less with that of the external solution (Table XI). The osmotic value of the secreted fluid, however, can be considerably higher, about 0.1 M sugar, than that of the expressed sap of the leaf. This proves that osmotic work must be done to keep the secretion going.

The apertures in the cutinized outer wall of the secretion cells enable fluid to leave the cytoplasm. It is assumed that sap will be given off from the secretion cells under the influence of the turgor pressure of these cells. The cytoplasmic fluid is not likely to flow out quite unchanged. Great molecular substances will naturally remain in the cytoplasm. NaCl is secreted in any case and in some cases organic substances are likely to be secreted as well. Ruhland states that 25 to 40 % of the dry matter of the secreted fluid of *Statice gmelini* may be attributable to organic substance. With leaves on destilled water he obtained secretion for 34 days. The secreted amount of fluid was then reduced to one fourth, the concentration of the dry substance being reduced to less than one third. If the water in his experiments has been changed, we may assume that after a day or two the leaves did not give off any more NaCl. The secretion, however, continued, though the rate decreased, the concentration being reduced as well. This would indicate a secretion of organic substances. The results of table XIV show that also in the case of *Limonium latifolium* a small quantity of other substances than NaCl may be found in the secreted fluid. We did not find, however, any secretion of fresh leaves of *Limonium latifolium* unless NaCl was administered. As far as we know there is no exudation of pure water. The subject deserves to be further examined especially in *Statice gmelini.*

Though the osmotic concentration of the sap in the gland cells cannot be directly determined, it can be estimated since the osmotic concentration of the secreted fluid must be equal to or somewhat smaller than the osmotic concentration of the sap of the gland cells. This will depend on the permeability of the plasm in the pores. The difference is caused by not exuded substances and will be relatively small when salt is present in high concentration.

The osmotic concentration of the sap equals the suction pressure plus the turgorpressure in the gland cells. In order to produce a sufficient turgorpressure the osmotic value of the gland sap has to be considerably higher than that of the external solution. The turgor can be reduced by enhancing the osmotic value of the external solution. A restoration of the turgorpressure needs an uptake of osmotically active substances in the gland cells till their suction pressure has become higher than that of the external solution.

It appears from Table XIV that the quantity of sap exuded is reduced by enhancement of the osmotic concentration of the external solution and that secretion is continued after a considerable increase of the osmotic concentration of the sap in the gland cells, which results in a higher NaCl content and a higher osmotic value of the secreted liquid. These experiments are in accordance with the assumption that the secretion is dependent on the turgorpressure of the gland cells. We have already discussed that the amount of secreted fluid is independent of the salt concentration of the gland sap (cf. p. 326). Seeing the NaCl concentration in the gland cell may vary, it is evident that the sap expressed under the influence of the turgorpressure may also be richer or poorer in NaCl.

Ruhland had already shown that secretion can take place against a fairly high pressure from outside, so that a coat of wax put on the surface of the leaf is pressed upwards. From a comparison of the anatomical structure of the glands of *Limonium* with those of *Spartina Townsendii* ARISZ (1955) has drawn the conclusion that the fluid is squeezed out by the pressure in the gland cell. In *Spartina* the aperture through which the sap exudes lies in the wall of an adjoining epidermal cell. Here the pressure in the gland cell must be transferred through the symplasm to the epidermal cells.

All these data indicate that not the exudation of the liquid from the cytoplasm is the energy requiring process in the secretion but keeping up the pressure in the gland cells by introducing substances into these cells.

The problem therefore is how this pressure and the salt concentration in the gland cells is being maintained and regulated. An active mechanism must carry a solution of water with salts and organic substances into the gland cells. A mechanism that carries water and dissolved substances simultaneously is unknown. It should, therefore be considered whether the introduction of salt and organic substance or that of water is the energy requiring process.

Processes of active salt uptake are well known. If by an active uptake of substance the pressure in the gland cells is maintained, the production of sap by the gland cells will take place at a constant rate. In leaves placed on water, the NaCl concentration of the secreted sap is reduced, the amount of sap being as great as in leaves on a salt solution, which have a much higher NaCl concentration of the secreted sap. This must be due to the fact that in both cases the pressure in the gland cells regulating the amount of fluid secreted, is equally great.

Whereas in leaves rich in NaCl especially NaCl will be pumped into the gland cells to keep the pressure up, in leaves on water the pressure is likely to be maintained by introduction of partly other substances than NaCl especially when these other substances get lost in the secreted fluid.

Temperature, light and inhibitors affect this active process that regulates the pressure in the gland cells and together with it the strength of outflow.

Some investigators have assumed an active water uptake into the cell. An efficient water uptake into the cell requires a membrane that can only be passed with the aid of an energy requiring process. If for the glandular action we assume as only active process an active water uptake into the gland cells, it is hard to understand how owing to temperature, light and inhibitors more fluid can be secreted without the concentration of the secreted fluid being reduced. This requires a simultaneous active salt uptake, organized in such a way that it is equally influenced by the factors mentioned as the active water uptake. The active salt uptake, however, would make the active water uptake superfluous.

Until we have more data at our disposal, it seems to us that the above conception of the mechanism as a system that carries salts into

the gland cells actively and by doing so causes a pressure in them resulting in an expression of sap from the pores, is the most acceptable. A definite opinion on the secretion mechanism can only be given, when the biochemical processes on which it is based, are known.

Here we may refer to the correspondence of this process with the secretion of a salt solution to the vessels with the bleeding of the root, discussed by ARISZ (1955).

SUMMARY

The concentration of NaCl in the external solution during the pretreatment influences the secretion by the salt glands. A pretreatment with 2 % to 3 % is most favourable, then both the amount and the concentration of the secreted fluid and also the NaCl concentration in the leaf are greatest (Table I).

The rate of secretion is constant during the first few days or is reduced (Tables III, IV, V). The amount of secreted fluid is usually somewhat higher on water than on salt owing to the lower osmotic value of the external solution.

The NaCl concentration of the secreted fluid may either increase or decrease during the secretion on a salt solution. With secretion on water there is always a decrease.

When exposed to light the amount of secreted fluid increases. The concentration of the secreted fluid does not change (Table VI).

If temperature is raised from 5 to 25° C the rate of the secretion of fluid increases 7 times. The concentration of the secreted fluid remains fairly equal, unless the secretion takes place on water (Table VII). Oxygen is required for the secretion.

In a low concentration inhibitors increase the rate of the secretion of fluid, in a high concentration they inhibit it. The NaCl concentration of the secreted fluid remains unchanged (Tables VIII and IX, Fig. 2).

The osmotic value of expressed leaf sap varies. The average osmotic value is equal to the osmotic value of the medium (Table XI). The osmotic value of the sap secreted by the gland is higher than the osmotic value of the expressed leaf sap (Table XI). For the secretion osmotic work is done.

The osmotic value of the secreted fluid, computed as NaCl solution, corresponds with the NaCl concentration of the secreted fluid (Table VI). In an other experiment (Table XIV) with secretion on sugar plus salt solution it was greater than the NaCl concentration of the secreted fluid. This indicates the presence of other substances in the secreted fluid. Increase of the osmotic value of the external solution:

(A) by *sugar* (Tables XII, XIII, XIV) decreases the amount of secreted fluid and increases the NaCl concentration and the osmotic value of the secreted fluid.

(B) by NaCl (Table XV) alters the amount of secreted fluid but little. On addition of too much diluted salt solutions or water there is a decrease of the amount of secreted fluid. The NaCl concentration in the secreted fluid increases. The total loss of Cl has increased.

(C) by $MgSO_4$ (Table XV) gives a reduction of the amount of secreted fluid and an increase of the NaCl concentration of the secreted fluid. The total loss of Cl has decreased.

The mechanism of the secretion may be considered as a system that introduces salts actively into the gland cells and by doing so causes a pressure that results in a squeezing of sap through the pores out of the gland cells. The amount of expressed sap depends on the pressure in the gland cells. The latter may be osmotically influenced by the external solution.

The composition of the expressed sap depends on the substances accumulated in the gland cells and on the permeability at the pores.

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