Summary.

1. A constant concentration of a polar non-electrolyte (e.g. an alcohol or keton) moves the viscosity KCl curve either to higher concentrations (the first terms of a homologous series) or to lower concentrations (the higher terms of a homologous series).

A middle term may show a transition character: in smaller concentrations it corresponds with the higher terms of a homologous series, in larger concentrations with the lower terms.

2. While the viscosity curve is moving as mentioned in 1), the maximum of viscosity decreases, the inflexion point in the left ascending curve branch gets less pronounced or disappears, while the phenomenon of thread-pulling declines or stops altogether.

3. The influence at 3 different, but constant KCl concentrations of methyl- (1), ethyl- (2), n. propyl- (3), n. butyl- (4), n. amyl- (5) and n. hexyl-alcohol (6) on the viscosity and the thread-pulling was investigated more in detail.

4. The influence on the viscosity still depends on the selection of the KCl concentration, being evident from 1) and 2).

At a KCl concentration before the inflection point. 6, 5 and 4 do increase the viscosity until a maximum is reached and decrease afterwards. 2 and 1 only decrease the viscosity, while 3 acts as transition term.

At the maximum of the KCl viscosity curve all terms of the homologous series just decrease the viscosity.

At still higher KCl concentrations now 2 and 1 increase the viscosity to a maximum and 6, 5 and 4 decrease, while 3 acts as transition term again.

5. The concentrations at which an alcohol acts on the viscosity (either increasing or decreasing) or on the phenomenon of threadpulling resp. (either stimulating or suppressing) is the smaller the more carbon atoms the alcohol contains.

Botany. — *Uptake and transport of chlorine by parenchymatic tissue of leaves of Vallisneria spiralis.* I. *The active uptake of chlorine.* By W. H. ARISZ.

(Communicated at the meeting of October 25, 1947.)

§ 1. *Introduction.*

The phenomenon of the active uptake of substances in plant cells has been extensively investigated on various objects. Many investigators have done this on disks of storage organs. Besides various advantages these objects have this disadvantage that the uptake is not a normal phenomenon in their case, but takes place as a result of wounding and regeneration. Therefore the uptake which is found here and which is inherent with the formation of new protoplasm and the protein metabolism departs in some respects from the normal uptake. STEWARD's generalization that only very active cells having the capacity for further growth show an active uptake seems premature.

Others have investigated the process of uptake in unicellular seaweeds which are much less apt to take up substances actively, such as Valonia and various Characeae as Nitella.

The greater number of researches, however, has been made with the roots of higher plants, in which an uptake occurs which in some respects resembles the active uptake in storage tissues and unicellular sea weeds. It has appeared, however, that this uptake is of a complicated nature because in their case we have not only to deal with the uptake by the root cells, but also with the transport of salts to the xylem. Therefore it is important that there is a fourth group of objects, the leaves of water plants, in which the problems are in some respects a little simpler.

Especially Elodea has served as an object for investigations.

In our own researches Elodea gave no distinct result, while with Vallisneria the results on uptake of salts proved to be much more suitable for analytical purposes. Besides this object has the advantage that not as in the case of Elodea, leaves and stalk were examined at the same time, but only the very homogeneous leaf tissue. For these long leaves it is moreover possible to trace the transport of the absorbed substances in the leaf. As far as I know, these leaves have not been used in absorption- and transport experiments, since BIERBERG worked with them in 1909 in a research on the influence of protoplasmic streaming on the transport.

The last ten years a series of researches have been made in Groningen on Vallisneria, in which the details of these processes have been extensively examined. As early as 1932 Miss A. KOK of Groningen had repeated BIERBERG's investigation on the uptake of Lithium salts and she had also examined the transport of caffeine. Next followed extensive quantitative

researches by ARISZ and OUDMAN in 1937 and 1938 into the uptake and the transport of asparagine and caffeine, in which it was found that absorption and transport of asparagine are active processes.

By ARISZ and VAN DIJK (1939) and ARISZ (1943) the details of the process of asparagine absorption were further determined. After that a research on the uptake of cat-and an-ions of anorganic salts was started, of which the results of the uptake of chlorine are summed up below.

Vallisneria spiralis can be cultivated in a hothouse all the year round. The leaves are so long that pieces of 30 cms. or longer can easily be cut off and can be divided into smaller pieces. The leaves grow only in their basal parts. Those parts which are used in the experiments do not display any further growth. A disadvantage of Vallisneria is that growing it requires much more care than the growing of Elodea and that only material cultivated in favourable circumstances in a suitable exposure to light can be used for these experiments. In discussing the method in § 2 the most important items which have to be observed in making the experiments, will be treated extensively. Another disadvantage of Vallisneria is the presence of big intercellular ducts which though they don't constitute an open communication, because of the presence of septa cf. A. KOK, may give rise to difficulties, when as a result of inefficient technics external liquid enters them. This namely takes place in the dark when the surrounding cells withdraw oxygen from the intercellulars, which are for a great part, filled with it. As a result water is being sucked up in the intercellular spaces. The infiltration is injurious to the leaf and causes a quicker dying off, but in addition, if the external solution is concentrated, the infiltration of this solution into the intercellular spaces may cause important errors, because of its giving the impression that the substances have been absorbed by the tissue.

If however, diluted solutions are used as is the case for this publication, this phenomenon need not be considered, provided the leaves continue to stay in a good condition. This obtains for all solutions more dilute than 1/50 to 1/100 mol. Vallisneria leaves have a number of vascular bundles running parallel in the length of the leaf. They consist almost entirely of parenchymatic cells. Vessels do not occur. This renders these leaves particularly suitable for experiments to investigate into the transport in parenchymatic tissue.

For the experiments and the analyses I received the valuable aid of the analysts Miss J. VAN DER SCHANS, Miss E. BOSMA and Miss H. MEESTER, for which I wish to express my indebtedness in this place.

§ 2. *Method.*

In this publication the uptake of Cl ions is exclusively discussed. These were determined by VOLHARD's method in about 200 mgrs fresh weight, while the analysis figures always refer to a certain size of leaf surface, mostly 19.2 or 20 cms with a width of 4 mms.

Some investigators have preferred examining Br ions, therefore something has to be said here about the choice of chlorine ions. Since HOAGLAND and STEWARD showed in a convincing manner that the uptake of Br ions is more complicated than that of Cl ions, because simultaneously with the uptake of Br a loss of Cl occurs, the use of Cl ions has to be preferred, if one wants to study the process of active uptake, as in that case the interchange of Cl and Br would only give an undesirable complication.

The Vallisneria plants were grown in concrete tanks measuring 200 to 58 cms, with a depth of 50 cms. Heating took place with hot water pipes and electric heating with an ozurite cable in the ground. The temperature was kept constant by a thermoregulator at 23° C.

Part of the year extra exposure was administered with a couple of 200 watt lamps during some hours in the evening. Of great importance is the water used for the experiments. On account of the well known fact that destilled water is injurious, various investigators have used tap-water or springwater. This surely gives much better results for keeping the cut leaves alive, but it should be remembered that the leaves might absorb salts from these highly diluted salt solutions and as a result the osmotic value of the cell-sap may rise considerably, as was first shown in this laboratory by Miss VAN SCHREVEN (ARISZ 1943).

For the experiments made water destilled over glass was used. All the experiments were made in a room of a constant temperature usually at 25° C.

We must refer to a previous publication (ARISZ and OUDMAN 1937) in which the division of material into smaller pieces and the composing of series from these was communicated. Owing to the fact that they contain pieces of different leaves, taken from different parts of the leaves, these series are very homogeneous with each other, so that the results of the chemical analysis show a very slight variability.

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 into smaller pieces and put in destilled water in series. The pieces of one series are sewn into a bit of tulle, so that they don't overlap. The tulle is weighted so much on one side that it floats in the solution in a vertical plane. All leaf-lengths can then be exposed to the sideways entering light of an electric lamp. Both during the preliminary treatment and during the uptake the leaf lengths were put in tumblers or in vessels with bottoms of sintered glass, a good aeration being seen to.

Regarding the rather great differences in the amount of the uptake in identical experiments it must be emphasized that conclusions must be based on the comparison of different treatments in one and the same experiment.

§ 3. *Influence of wounding.*

In experiments on uptake and transport of asparagine by ARISZ and

OUDMAN 1937, it had already appeared, that leaf lengths of which a small part had been brought in touch with asparagine, while the rest of the leaf had to get it from this small leaf length, took up more asparagine according as the free part was longer. This phenomenon was met again in the experiments on the uptake of chlorine and will be discussed when we deal with the transport. It was of essential interest to know what this influence of the free leaflength could be based upon. Presumably this could at least partly be due to the wounding, owing to which a longer leaf length would be less impeded in the uptake by the wound-stimulus, as the wounds inflicted lie farther apart. This phenomenon could better be investigated in this way that the uptake of leaf lengths of a different size which could absorb salt over their whole length, were compared and it was traced, whether the uptake calculated for an equal leaf surface, was always equally great. If the wounding should affect the uptake, it would have to appear from a slighter relative uptake by short leaf lengths.

To ascertain this the following experiment was made. Leaf lengths of 7.5 cms, 2.5 cms and 0.83 cm were brought into a solution of 1/1000 mol. KCl with $CaSO_4$. After 24 hours it was determined how much Cl had been absorbed. For this experiment 8 leaves of a length of 30 cms were used. These were cut to a uniform width of 4 mms and divided into 4 pieces of 7.5 cms. By combining in a series 8 leaf lengths taken from the 8 different leaves but from a different place, 4 comparable series were obtained. In two of these series the leaf lengths were further divided into 3 lengths of 2.5 cms, so that the experiment was made with:

A. two series, each of 8 leaf lengths of 2.5 cms = 20 cms, which were analyzed at the beginning of the experiment.

B. two series of 8 leaf lengths of 7.5 cms. After the uptake these leaf lengths were cut into 3 lengths of 2.5 cms and the Cl was separately determined in the 3 zones. Here too 20 cms of leaf is available for each analysis.

C. two series, each of 8 leaf lengths of 2.5 cms = 20 cms.

D. two series each consisting of 24 lenghts of 0.83 cm, i.e. likewise 20 cms per series.

The table gives the amount of chlorine in each of the series B, C and D after a 24 hours'uptake. The difference with the series sub A gives the increase of chlorine per 20 cms leaf length.

Short leaf lengths take up considerably less than longer ones. This is due to a wound influence. This follows from the analysis of the 3 different zones of the leaf lengths of 7.5 cms, of the series *B 1* and *B 2*. The marginal zones take up considerably less than the central part. As this wound influence could not be prevented, it was desirable, to discover if the wound influence grows weaker in the course of time. For this purpose the cut leaf lengths were put in destilled water for a different length of time. The

TABLE I. Absorption of chlorine by leaves of different length. Uptake during 24 hours from a solution of 0.001 mol KCl with $CaSO_4$, exposed to light (200 Watt at 50 cm). Cl content per 20 cms leaf length.
B_1 B_2 leaf length 7.5 cms C_1 C_2 2.5 cms D_1 D_2 0.83 cm

	γ Cl	uptake γ Cl	average uptake per 20 cms leaf length γ Cl
A_1 control series 2.5 cms	432		
A_2 " " " "	437		
B_1 first zone 2.5 cms	568	134	
second zone 2.5 cms	611	177	146
third zone 2.5 cms	561	127	
B_2 first zone 2.5 cms	571	137	
second zone 2.5 cms	614	180	148
third zone 2.5 cms	561	127	
C_1 leaf length 2.5 cms	532	98	
C_2 leaf length 2.5 cms	536	102	100
D_1 leaf length 0.83 cm	504	70	
D_2 leaf length 0.83 cm	512	78	74

following experiment gives the result of a preliminary treatment of 2, 4, 7 and 24 hours on the uptake of 1/1000 mol KCl with $CaSO_4$ in the light at 25° C. In this case the series consisted of 8 leaf lengths of 2.5 cms each. We only mention the increase of chlorine per series of 20 cms total leaf length.

TABLE II. Influence of duration of pretreatment in destilled water on the uptake of chlorine.

Preliminary treatment no.	uptake of Cl in γ. 98
2 hours in destilled water	120
4 hours in destilled water	131
7 hours in destilled water	142
24 hours in destilled water	246

From this it appears that preliminary treatment in destilled water has the desired effect and that after 24 hours the inhibition due to wound-influence has disappeared for the greater part.

After this the experiment with bits of leaf of various lengths was repeated, now after a 24 hours'preliminary treatment in destilled water.

Table 3 gives the results of this experiment. It appears that lengths of 2.5 cms and 7.5 cms give the same results. Besides the difference between

TABLE III. Absorption of chlorine by leaves of different length after pretreatment 24 hours in destilled water. Uptake during 24 hours from a solution of 0.001 mol KCl with $CaSO_4$, exposed to light (200 Watt at 50 cms' distance). Cl content per 20 cms leaf length.

B_1 B_2 leaf length 7.5 cms C_1 C_2 2.5 cms D_1 D_2 0.83 cm

	γ Cl	uptake γ Cl	avarage uptake per 20 cm leaf length γ Cl
A_1 2.5 cms no uptake	404		
A_2 „ „ „ „	401		
B_1 first zone 2.5 cms	644	241	
second zone 2.5 cms	640	237	238
third zone 2.5 cms	640	237	
B_2 first zone 2.5 cms	644	241	
second zone 2.5 cms	648	245	242
third zone 2.5 cms	644	241	
C_1 leaf length 2.5 cms	640	237	234
C_2 leaf length 2.5 cms	634	231	
D_1 leaf length 0.83 cm	564	161	154
D_2 leaf length 0.83 cm	551	148	

marginal and central zones has disappeared with the 7.5 cm leaf lengths. In the 0.83 cm leaf lenghts the uptake after a 24 hours'preliminary treatment is still considerably slighter. From these and other experiments not mentioned it may be concluded that a 24 hours'preliminary treatment in destilled water eliminates the wound effect sufficiently for leaf-lengths of 2.5 cms and longer. From the result of a great number of comparative experiments it has appeared that the wound effect not only influences the strength of the uptake, but also influences the transport. To this we shall revert on discussing the transport.

§ 4. *Experiments on the uptake of chlorine.*

A. *from solutions.*

The experiments were partly made in 1944, when the wound effect had not yet been investigated, partly in 1946 and 1947, when this could be entirely taken into account. The results of the two series are in perfect agreement. This is partly due to the fact that the wound effect is not always so great as in the above experiments, but also to the fact that the wound effect only impedes the processes of uptake without altering them in

essence. Initially experiments were made on the uptake of chlorine from aqueous solutions of various chlorides. Various chlorides, especially LiCl and Mg Cl_2 but in some experiments NaCl as well were not absorbed at all in a concentration of 1/320 mol. On the contrary a loss of chlorine from the tissue was often stated which indicated the toxicity of these salts. In more resistant leaves the loss of chlorine changed into an uptake. $CaCl_2$ was taken up most easily, KCl considerably less. Since this indicated a toxicity of the salt solution and as in lower concentrations such as 1/1000 mol toxicity was somewhat less than in higher concentrations, though the results remained variable, we proceeded to operating with a mixture of potassium and calcium ions to balance the toxicity of the cations.

Seeing the Vallisneria plants were grown in well-water and in the tanks a continual evaporation of water occurred the quantity of salts and particularly of calcium in the water increased during the growing. It is known that under these circumstances Vallisneria leaves depose $CaCO_3$ on the surface exposed to the light. Older leaves were indeed often covered with $CaCO_3$. Though usually younger leaves were used for the experiments, they can also be covered more or less with $CaCO_3$, so that on our making experiments in water containing carbonic acid, $Ca(HCO_3)_2$ will also have been present. This may account for the fact that in some experiments an equal uptake from KCl as from a mixture of KCl and $CaSO_4$ was found.

Besides the toxicity of the cations the destilled water used may have its influence. It has repeatedly appeared that in solutions with ordinary destilled water no uptake but exosmosis occurred. That is why, as already stated above, in all experiments water destilled over glass has been used. The solutions of KCl and $CaCl_2$ did give better results yet no satisfactory ones. By adding $CaSO_4$ instead of $CaCl_2$ a solution was obtained which always gave favourable results. As a rule a mixture of KCl and $CaSO_4$ (747 mgs KCl + 368 mgs $CaSO_4$ 2 aq in 1 L water) was used and this mixture was diluted if required. This gave a ratio of potassium and calcium ions of 62 : 18 and of chlorine and SO_4 ions as 63.5 : 36.5. Seeing in this case the issue is the absorption of chlorine, the strength of the concentration is indicated in mol KCl. With this combination of salts a great number of experiments have been made, the results of which were very regular, which makes them seem sufficiently reliable.

Influence of deprivation of oxygen.

Some experiments have been made to trace whether in an anaerobic medium chlorine was taken up. Of course these experiments had to be made in the dark, as in the light oxygen is set free during assimilation. Never was any absorption found in these experiments as a rule loss of chlorine occurred, because the leaves started showing dying off phenomena.

Influence of salt concentration.

Table 4 contains a number of data on experiments in which the in-

TABLE IV. Influence of concentration on the uptake of chlorine by leaves of Vallisneria from KCl solutions containing $CaSO_4$. Experiments in the light 200 Watt at 50 cms, 25° C, 24 hours. The figures give the increase in Cl concentration in milli mol of 8 leaf pieces of 2.5 cms length and 4 mm width, A and B with a short pretreatment, C and D with pretreatment during 24 hours in destilled water.

Chlorine conc. outer solution in milli mol	uptake of Cl in milli mol				accumulation factor	
	A	B	C	D	C	D
4	30		35		9	
2	29		30		15	
1	29		33		33	
1/2	29	24	32	31	64	62
1/4	28	22	27	28	108	112
1/8		15		25		200
1/16		9		17		272
1/32		2		11		352

fluence of the concentration of chlorine ions in the solution on the uptake has been traced. All the experiments were made in the light, those of 1947 after a 24 hours'preliminary treatment in destilled water in the light. The data are in perfect agreement: from 1/32 to 1/2 millimol there is a rather strong increase in the uptake, about proportional to the logarithm of the concentration. When, however, results of various experiments are compared, rather great differences may appear. This is due to the fact that the condition of the plants at the beginning of the experiment greatly influences the uptake. This also appears from experiments made with a different preliminary treatment. An influence on the uptake is exercised by the strength of the exposure, the supply of oxygen, the composition of the air used for aeration and the concentration of hydrogen ions of the medium.

Accumulationfactor.

From the data of table IV an accumulation factor can be evaluated. When we suppose that the chlorine taken up is solved in the water present in the tissue we get an approximative value of the inner concentration. The accumulationfactor that is the relation between the concentration of the chlorine in the tissue and in the outer solution increases from 9 at a concentration of the outer solution of 4 millimol Cl to 352 at a concentration of 1/32 millimol Cl.

Influence of exposure during the uptake.

After a 24 hours'preliminary treatment in the light in destilled water, the uptake from 1/1000 mol KCl + $CaSO_4$ in the light was 157 γ Cl, whereas in the dark it was 16 γ Cl. As a source of light during the preliminary treatment a 100 watt lamp was used at a 50 cms'distance (6450 lux), and during the uptake a 200 Watt lamp at 50 cms (12900 lux). Also the

intensity of the light has its influence. On this subject only some orientating experiments were made.

TABLE V. Influence of light-intensity on the uptake of chlorine from 1/1000 mol. KCl with $CaSO_4$. Time of uptake with A 28 hours, with B 26 hours. Light source 200 Watt at 50 cms' distance.

at a distance of	A	B
50 cms	208	215
100 ,,	154	190
200 ,,	90	115

From these data it is apparent that the intensity of the light during the uptake has a considerable influence.

Analysis of the influence of light on the uptake of chlorine.

In this series of experiments the effect of light on the uptake was further examined. Seeing light also gives photosynthesis, it was interesting to know whether light in an environment free from carbonic acid also effects the absorption of chlorine. The result of these experiments has been given in table 6. An environment free from carbonic acid was obtained by letting

TABLE VI. Analysis of the influence of light on Cl-accumulation. Absorption 24 hours of 0.001 mol KCl with addition of $CaSO_4$ at 25° C. A and B in the light, C and D in the dark. A and C in solution aerated with common air. B and D in an aerated solution deprived of CO_2. Experiment V pretreated 24 hours with destilled water, exposed to light and aerated with CO_2 free air.

	aeration	uptake in γ Cl				
		I	II	III	IV	V
A. light	with air	118	133	296	134	264
B. light	without CO_2	122	188	360	209	292
C. dark	with air	13	1	97	28	
D. dark	without CO_2			117	39	

air free from carbonic acid bubble through the solution before and during the experiment, so that for photosynthesis at most the small quantity of carbondioxyde originating from the respiration was available. In addition in some experiments the solution had been boiled beforehand and cooled after closing off with a tube containing soda-lime. It is evident that light without carbonic acid has the same effect as light with carbonic acid. This proves that the effect of light on the active uptake of chlorine is not connected with carbondioxide assimilation. Besides it appears from table 6 that the uptake in an environment free from carbonic acid is considerably greater than in a solution containing carbonic acid. This also holds good for experiments in the dark, only the level is much lower in this case. It seems probable that in case of withdrawal of carbonic acid the stronger uptake may be the result of the higher pH in the medium.

Influence of pH on the uptake of chlorine.

Therefore the influence of the concentration of *H* ions on the uptake was examined. The experiments were made in a continuous artificial light. In these experiments the pH of the unbuffered solutions decrease during the experiment. This may be due to an increase of carbonic acid in the medium. Aeration was brought about with common air. The change is somewhat slighter if during the experiment aeration takes place with air free from carbonic acid. In these unbuffered solutions the optimum has not always been found at the same level. It lies at about pH 6—7. Evidently pH 4.5 is detrimental, so that a loss of chlorine occurs due to exosmosis, likewise pH 9 is toxic. It is therefore not impossible that on aeration with air free from CO_2 the uptake is greater owing to the fact that the pH lies nearer to the neutral point. In the same way aeration during the preliminary treatment with air free from CO_2 may give a stronger uptake than aeration with air containing CO_2.

Preliminary treatment.

The preliminary treatment is of great importance, which appears from some experiments on the influence of light during this period on the strength of a later uptake. In one experiment e.g. the uptake from 0.001 mol KCl + $CaSO_4$ during 24 hours in the light, was 158 γ Cl, if during the 24 hours'preliminary treatment the leaflengths were exposed, but only 76 γ Cl if they were kept in the dark. Therefore in experiments with preliminary treatment the objects were always exposed to the light in this period. Also the intensity of the exposure during the preliminary treatment has its influence. Aeration during the pretreatment with carbondioxide free air and the withdrawal of carbondioxide formed by the tissue does not prevent this favourable influence on the following uptake. So this effect has nothing to do with photosynthesis of carbohydrates.

It seems interesting to know if an exposure to light during the pretreatment has the same effect as an exposure during the uptake. Both processes have in common that the influence of light is not through products of carbon dioxide assimilation. Still it appears that both effects are not identical because a leaf pretreated in light must be exposed during the absorption as well to get a normal uptake. It was ascertained wether the effect of light during the pretreatment influenced the uptake only in the next few hours following the pretreatment or that it lasted for a longer time. The results of two experiments are given in table 7. In each experiment three series with different pretreatments were exposed at the same time in a 0.001 mol KCl solution, and the uptake was determined after 6 and 24 hours. The uptake in the last 18 hours could be evaluated from these data. It is apparent that though the uptake in the first six hours depends on the pretreatment, the difference in strength is also present in the following hours. So the uptake in the second period was e.g. in experiment A 160 γ after exposure during the pretreatment to strong light, 105 γ to less strong

TABLE VII. Influence of exposure to light during pretreatment on the uptake of 0.001 mol KCl + $CaSO_4$ in the light, 25° C. Two experiments A and B. The uptake is determined after 6 hours and after 24 hours.

Pretreatment	uptake in γ Cl after 6 hours		uptake in γ Cl after 24 hours		uptake in γ Cl in the last 18 hours	
	A	B	A	B	A	B
24 hours exposed 12900 lux	144	146	304	250	160	104
24 hours exposed 6450 lux	122	108	227	193	105	85
24 hours in the dark	74	57	115	106	41	49

light and 41 γ when the pretreatment was in the dark. This means that the effect of the illumination during the pretreatment influenced the uptake all the time.

In another experiment the uptake took place in the dark after the same different pretreatments. In all cases the uptake was now only slight.

These results indicate that during the pretreatment in light a substance e.g. a sensitizer may be formed which favours the process of uptake in the light. The present data are not sufficient to give a complete analysis of this phenomenon.

B. *Uptake from agar.*

For all experiments in which it was necessary that only part of a leaf length should take up salt in which case it can be traced if transport takes place to the other zones of this leaf length, a 2 % agargel was used as a medium in which the salts were dissolved. For these experiments the agar was not specially purified, because in such a purification decomposition products of proteins are apt to be formed, of which a complicating action on the plasm is to be expected. So it appeared for instance, that if so called purified agar was used, the uptake in the dark was sometimes much stronger. As unpurified agar contains some chlorine, a leaf could already absorb some Cl from the blank agargel in 24 hours. This amounted to about 5—10 γ Cl in 24 hours per 20 cm leaf-length. It did not present difficulties however, that in these experiments, in which chlorides were added to the agar anyhow, a slight amount of chlorine was already found in the agar. The above experiments on the influence of different factors were repeated with lengths in agar and results were found in perfect agreement with those already stated. With regard to the wound stimulus as a result of cutting it was found that material which had had a 24 hours'preliminary treatment in destilled water in an exposure to 100 Watt at a 50 cms'distance, absorbs in 24 hours relatively more in leaf lengths of 7.5 cms than in leaf lengths of 2.5 cms, viz. 315 γ Cl against 286 γ Cl. This indicates that in this experiment with leaf lengths of 2.5 cms after 24 hours the wound

stimulus had not quite disappeared yet. This is in accordance with the experiments on the uptake from solutions.

With these experiments the difference between uptake in the light and in the dark is less pronounced than with experiments in solutions, because the uptake in the dark is slightly greater. This may very well be due to the presence of disintegration products in the agar. So in an experiment with leaf lengths of 7.5 cms for the uptake in the light in the first zone of 2.5 cms 229 γ was found, in the 2nd zone 233 γ and in the 3rd zone 218 γ Cl, whereas in the dark the amounts were 90, 83 and 79 γ respectively.

§ 5. *Exosmosis of absorbed chlorine.*

For the asparagine uptake there was formerly made an extensive study of the phenomenon of exosmosis of the first absorbed asparagine. Whereas on transport to an anaerobic medium no exosmosis was obtained, after transport into a fresh solution an exosmosis of part of the first absorbed asparagine took place for some hours. After that the leaf recovers itself and reabsorption takes place. This phenomenon is closely connected with the sensitiveness of the material. Resistant material, such as has been used for these experiments with salts, does not show exosmosis at all or in a much slighter degree. In the experiments on exosmosis of first absorbed salts no loss has been observed in undamaged tissue. Transport from light into dark or from the salt solution into destilled water had no influence at all. As already stated, an exosmosis was obtained with extreme pH. Neither does oxygen withdrawal in itself bring about exosmosis. If, however, the leaves continue under these unfavourable circumstances, in the dark for a long time, a loss is noticed right enough. We must therefore conclude that under normal circumstances salts which have once been absorbed are not returned to the environment again.

§ 6. *Osmotic value of the cell sap.*

It is essential to know whether the salts absorbed, of which here only the Cl ion was determined, get into the cytoplasm or are taken up partly or entirely in the vacuole. If they get into the vacuole they may be found there bound or absorbed or continue free. Only in the latter case they will cause an increase of osmotic value. If therefore after the uptake an increase of the osmotic value of the cell-sap is found, it may be concluded that the ions get into the vacuole and are present there in a free condition. The result of the experiments made to ascertain this fact are very convincing. The osmotic value of the vacuole at limiting plasmolysis has increased considerably after the uptake. The method that has to be applied to demonstrate the uptake of salt in the vacuole is simple and corresponds entirely with a similar research on the uptake of asparagine (ARISZ and VAN DIJK 1939). In a control series the osmotic value of a sucrose solution giving limiting plasmolysis is ascertained at the beginning of the experiment and in the experimental series which has absorbed Cl for 24 hours from a

solution of 1/1000 mol $KCl + CaSO_4$ this value is determined at the end of the experiment. As a plasmolyticum sucrose was used. In an experiment on uptake 217 γ Cl was found, while the osmotic value of the epidermal cells at limiting plasmolysis increased from 0.32 to 0.38 mol sucrose. In another experiment the uptake was 225 γ Cl and the increase from 0.30 to 0.38 mol. So the increase of the osmotic value is respectively 0.06 and 0.08 mol. If we consider the absorbed quantity of Cl dissolved in all the water present in the leaf length, this constitutes an increase of concentration of 0.031 mol and 0.032 mol. The quantity of water in the vacuole will of course be less than the total quantity of water present; moreover the salt will be dissociated. This renders it comprehensible that an even stronger increase of osmotic value was found (0,06 and 0,08 m instead of 0,031 and 0,032), but it indicates at the same time that the salt absorbed is found for the greater part free in solution in the cell sap. The chance that the very low concentration of the salt solution has caused metabolic processes, which should have brought about such an anatonosis of the cell-sap, may be considered out of the question.

§ 7. *Discussion.*

After that in some previous researches the active absorption of asparagine had been demonstrated, the uptake of salts, especially of chlorides, was further examined. As a result it was found that from a balanced salt solution the leaves of Vallisneria absorb chlorine. As the leaf lengths used in these experiments do not grow during the uptake we have here an active uptake which is independent of growth. The quantity absorbed depends on the concentration of chlorine in the external liquid, especially in concentrations lower than 1/4 millimol. The uptake is influenced by the pH of the solution, pH 4.5 and > 9 are detrimental and cause exosmosis of chlorine from the tissue. Exposure to light has a remarkable effect on the strength of the absorption. The stronger it is, the stronger is the absorption.

Light does not work photosynthetically here by forming carbohydrates, as it is also active when carbonic acid is not present in the medium. In literature various data are known on the fact that light affects the uptake of salts. In 1937 JÄRVENKYLA made an excellent summary of the extensive literature on the effect of light on the permeability of the protoplasm. In it he also treats the effect of light on the uptake in Nitella, Valonia and Elodea, found in 1923 and 1926 by HOAGLAND and collaborators, in 1934 by JACQUES and OSTERHOUT and in 1936 by INGOLD and points out that in these experiments light does possibly not affect permeability but processes of a different nature. Several of these investigators have connected the influence of light with photosynthesis. As it, however, appeared in our experiments with Vallisneria that in a medium free from carbonic acid, i.e. without photosynthesis light has the same influence, this conception cannot be accepted in the case of Vallisneria. PHILLIS and MASON (1937) found that cotton leaves only in the light and when supplied with oxygen,

absorb sugar from a sugar solution and form starch. This process also takes place in a medium free from carbonic acid.

From JÄRVENKYLA's publication and also from our own observations on Elodea cells it appears that the behaviour of various objects is different. Therefore it is advisable not to draw conclusions from the data given here for Vallisneria as to the behaviour of other objects. The problem will have to be carefully investigated for every ion and for every plant.

The process of absorption is an active process, as appears from the great sensitiveness of this process to outward circumstances. It is dependent on the presence of oxygen and on the temperature. Important is the great influence of the previous history. Wounding appears to have a rather strong effect due to which, especially in those parts of a leaf length which are nearest to the wound-edge, the processes of uptake are checked. This wound influence diminishes in course of time, so that in sufficiently great leaf lengths there is little to be observed of it after 24 hours. So to get a normal uptake it is necessary to apply a 24 hours'preliminary treatment in destilled water. Exposure, temperature, aeration during this preliminary treatment, they all have some influence on the strength of the ensuing uptake (Cf. p. 11).

It was possible to render it probable that the greater part of the substance absorbed gets into the vacuole. The increase in osmotic value at limiting plasmolysis tallies fairly well with the supposition that all chlorine absorbed gets into the vacuole. The cells do not return the absorbed chlorine to their environment. Neither through transport to destilled water nor through oxygen withdrawal from the medium, a loss was to be brought about. As soon, however, as injury was caused by toxic ions or by a too high or too low pH, exosmosis of chlorine could be shown. This proves that the protoplasm as a whole does not allow the chlorine ions to pass as long as it is in a perfectly normal condition.

Summary.

Leaves of Vallisneria spiralis take up Chlorine by an active process from balanced solutions containing KCl and $CaSO_4$.

The uptake depends on the presence of oxygen. It is influenced by exposure to light, by the pH of the solution, by temperature and by the pretreatment of the leaves. The influence of light is not indirect through the products of the process of photosynthesis but it is a direct effect on the processes occurring in the cytoplasm. From the cutting of the leaves ensues a wound-stimulus which checks the processes of active uptake. The normal condition returns after a prolonged stay in destilled water.

The increase of the osmotic value of the epidermal cells proves that the salts are accumulated into the vacuole.

Exosmosis does not take place from undamaged tissue. Under normal circumstances salts once absorbed remain in the vacuole. The protoplasm as a whole does not allow the chlorine ions to pass.

Zoology. — *The external shape as a specific character in Loxothylacus (Crustacea Rhizocephala).* By H. BOSCHMA.

(Communicated at the meeting of October 25, 1947.)

In a previous paper (BOSCHMA, 1940, Temminckia, vol. 5), in which the specific characters of a number of species of the genus *Loxothylacus* were described in some detail, especially with regard to variation, no mention was made of the macroscopical external peculiarities of the specimens. As it proved that the shape of the animals described in the cited paper may be characteristic for the species, some remarks are given here concerning their external appearance.

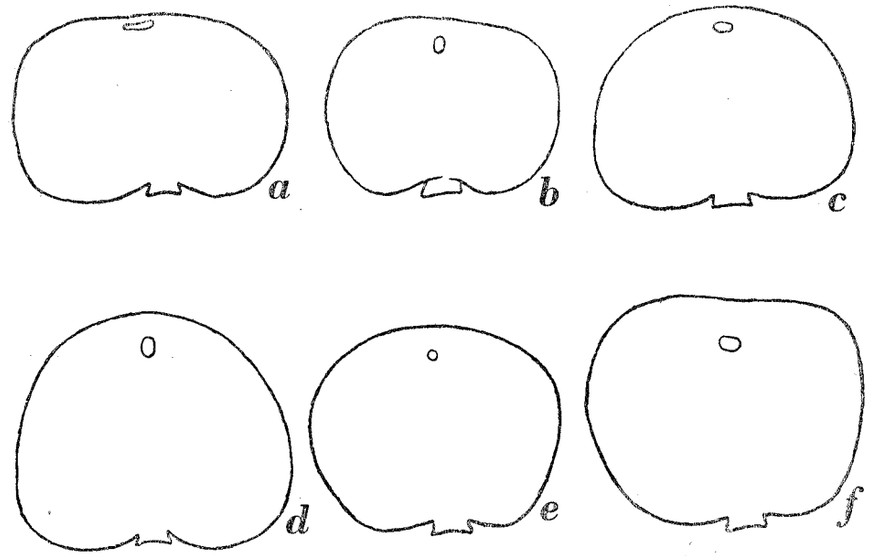

Fig. 1. Left side of six specimens of *Loxothylacus variabilis*. *a—d*, on *Chlorodiella nigra* from Koepang, Timor; *e*, on *Actaea rüppellii* from Mamoedjoe, Celebes; *f*, on *Actaea hirsutissima* from Taliaboe, Soela Islands. *a*, *c*, *d*, *e*, × 6; *b*, × 6½; *f*, × 5.

In *Loxothylacus variabilis* Boschma (fig. 1) the dorso-ventral diameter is slightly or appreciably larger than the antero-posterior diameter. The shape of the animals is elliptical or slightly panduriform or more or less reniform. The surface of the mantle does not possess any pronounced grooves or wrinkles. As a rule the mantle opening is rather narrow, it is found on the left side (the surface touching the thorax of the crab), not far from the anterior margin. This opening is very little conspicuous as it is