4. The elastic behaviour of maximum viscous KCl oleate systems is altered by alcohols. An alcohol acts in lower concentrations, the longer its carbon chain.

5. In the homologous series of the alcohol a change of action occurs with respect to the oscillation time. The first two terms increase, the 4th, 5th and 6th decrease the oscillation time.

n. Propylalcohol acts as transition term.

6. All six terms increase the damping.

7. Analogies to the influence of KCl and of alcohols on the viscosity of high viscous oleate systems and on the volume of oleate coacervates is briefly mentioned.

Botany. — *Uptake and transport of chlorine by parenchymatic tissue of leaves of Vallisneria spiralis.* II. *Analysis of the transport of chlorine.* By W. H. ARISZ.

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

§ 1. *Introduction.*

After it had appeared that leaves of Vallisneria when exposed to the light are apt to take up chlorine from highly diluted concentrations of balanced salt solutions, the transport of the absorbed chlorine ions in the leaf was examined. With the simple phenomenon that a leaf brought into a KCl solution absorbs chlorine, it is an obvious assumption that this is done by the epidermal cells, which subsequently transport the absorbed chlorine to the deeper lying cells. Though it is imaginable that the ions diffuse inwardly along the cell walls and consequently the more inward cells can also obtain salt from the external solution, such a diffusion in the cell walls will, if it is possible, proceed extremely slowly, because the concentration of ions in the medium is low, and with these submersed leaves no suction of water from the outside to the inside takes place, in consequence of which salts might be transported together with the absorbed water. It is quite certain that the subepidermal cells absorb chlorine as well, because they increase their osmotic concentration to the same degree as epidermal cells, as appears from plasmolysis experiments. How this transport takes place is still unknown and it is no simple thing to investigate this by means of a suitable experiment. This problem is much easier to be solved, when we bring a part of the leaf in touch with a salt solution and next try to find out whether transport is possible to other parts of the leaf which are not in direct touch with the salt solution. Such leaflengths must have obtained their increase in salt through transport in the leaftissue from the part that is in direct touch with the salt solution. Such a research has been made in my laboratory by Miss KOK for Li-salts and caffeine while ARISZ and OUDMAN examined the transport of asparagine and caffeine by bringing a small part of the leaf in touch with an agargel to which the substances had been added. In this way we get a purely local absorption of the substances by a certain part of the leaf and the transport to the rest of the leaf can be examined. As the agar used contained some chlorine the rest of the leaf was not put between agar, but between moist filter paper. This prevents transpiration in the free part of the leaf so that no suction of liquid from the part lying in agar can occur. This method of administering the salt solution locally in an agargel is (for experimental reasons) much to be preferred to the method of bringing a part of the leaf into a separate box filled with a liquid, because the proper closing

off of this box, is always attended with difficulties owing to leakage or injury of the leaf.

Two series of experiments were made. The first series took place in 1944. The second series in 1946 and 1947. The results of these two series are somewhat different. With the experiments of 1944 the influence of wounding caused by dividing the Vallisneria leaves into smaller parts, had not yet been investigated. As already discussed in the first part of this publication, the wounding has some influence on the uptake, but it is only of a quantitative nature. Leaves which are used for absorption experiments some hours after the wounding, show a fair absorption which under favourable conditions does not depart much from that of material subjected to a longer preliminary treatment. With the transport, however, deeper lying differences appear, so that it is necessary to treat the two experimental series separately. This is a remarkable example of the fact how experiments which quantitatively gave most reliable results, still suggest an incorrect interpretation of a physiological process, owing to the fact that a physiologic factor was still unknown.

§ 2. *Method.*

In the experiments made in 1944 leaf lengths of 45 mms, of a width of 4 mms were put with one end for a distance of 8 mms between two 8 mm wide strips of agar (2 %) to which 1/100 mol KCl had been added. In the later experiments of this series a mixture of KCl and $CaSO_4$ was used in which the toxicity of the ions had proved to be slight, as a rule in an 1/100 mol Cl concentration. For one series 24 leaflengths of 45 mms were used, which were divided into 5 pieces of 8 mms when the experiment was finished. Besides at the ends about 2 mms were cut off which projected from the agar, in order to keep the airpassages of the leaves open for the sake of a good aeration. 24 identical 8 mm pieces, i.e. 19.2 cms leaf length were analysed together. The free parts of the leaf lengths were either placed between moist filterpaper on both sides or laid on moist filterpaper with the lower side only, a glass plate being placed over the

TABLE 1.

Absorption of chlorine in the light by leaves of Vallisneria lying between sheets of moist filterpaper. 25° C.

Directly analysed γ Cl in 20 cms leaf	Analysed after 24 hours between filterpaper γ Cl in 20 cms leaf.
384	377
390	377
377	380
380	383
377	377
av. 381,6 ± 5,5	av. 378,8 ± 2.8

upper side. Controlexperiments showed that from filterpaper nothing is taken up (Cf. Table 1).

In the second series of experiments in 1946 and 1947 leaf lengths of 7.5 cms were used which after the uptake were cut into pieces of 2.5 cms. Series of 8 pieces of 2.5 cms were analyzed together.

The variability of the various leaflengths was eliminated by combining in one series pieces of various leaves taken at various distances from the top. To examine the effect of light on separate zones tinfoil boxes were used, in which the ends of the leaf-lengths lay side by side in one box, so that only the absorption zone was darkened for a distance either of 8 mms or in the later experiments of 25 mms. The remainder of the leaf-lengths was kept outside the box and could be placed into the light without the pieces in the box being exposed to the light. Through clefts in the side wall the leaf parts that had to be darkened, were put in the tinfoil boxes, the somewhat projecting cover above the cleft preventing a direct entering of the light through the clefts into the box. In another model of tinfoil boxes 4 zones of 8 mms or 2 zones of 25 mms of the leaves could be darkened, while only one zone was illuminated. The whole set up was put in closed glass boxes, the bottoms of which were covered with water, so that the air was saturated with water vapour. The experiments were made in a room for constant temperature at 25° C.

§ 3. *First series of experiments.*

In these experiments the leaflengths were put in water for only a few hours after cutting. For an experiment 12 leaves were used of 27 cms length, each divided into 6 pieces of 4.5 cms. 24 lengths of 4.5 cms are further divided into 5 parts and form 5 series, each of 24 pieces of 8 mms. These 5 series are used as a blank to determine the chlorine present, when the experiment is started. In addition there are two more series each of 24 pieces of 4.5 cms length, used for transport experiments. From these leaf lengths of 4.5 cm 8 mms are placed between agar strips, KCl + $CaSO_4$ being present in the agar. When the uptake is finished, the lengths are cut into 5 pieces of 8 mms and each time 24 identical pieces are analyzed together.

In the first series of experiments the difference in uptake and transport in a medium with and without oxygen was examined. In a medium free from oxygen there is neither uptake nor transport of chlorine.

A great number of experiments were made on the influence of an exposure to light on the uptake and transport of chlorine. In the experiments of table 2 the leaf pieces were exposed in their entire lengths, while the controlpieces remained in the dark. In this experiment, which just as those following was made with 1/100 mol KCl + $CaSO_4$ the uptake in the light was greater than in the dark. For a further analysis of this influence of light the experiments have been made in the above mentioned tinfoil boxes. From the result of these experiments it may be concluded

TABLE 2.

Influence of light on the transport of chlorine. One series of leaves in the light, the other one in the dark. Uptake in γ Cl from agar with 1/100 mol KCl with addition of $CaSO_4$ during 24 hours. 25° C.

	Increase of Chlorine in γ.	
	in the light	in the dark
First part of 8 mms in contact with agar 2 % containing KCl + $CaSO_4$	119	91
Second part of 8 mms	77	52
Third part of 8 mms	63	39
Fourth part of 8 mms	39	32
Fifth part of 8 mms	24	22

that the darkening in the tinfoil boxes has been sufficient. This appears from the following observation. In some experiments the first zone i.e. the *contactzone* was in the dark, the remainder of the leaf, which for convenience's sake we shall call the *free part* being put either in the light or in the dark. It now appears that the contactzone, which lies between the agarplates with the salt solution and which is in the dark, absorbs an equal amount no matter whether the free part is in the light or in the dark. Moreover it appears from tables 3 and 4 that exposure to light of the contactzone greatly affects the uptake. But as not any difference in uptake was found for the darkened contactzone, whether the free part is exposed to light or not, this proves that the contactzone receives no light on exposure of the free part; therefore the isolation in the tinfoil boxes is satisfactory.

TABLE 3.

Influence of an illumination of the contactzone. The free part of the leaves in the light. Uptake in γ Cl from agar with 1/100 mol KCl + $CaSO_4$ during 24 hours. 25° C.

		Increase of Chlorine in γ.			
		A		B	
	contactzone	dark	light	dark	light
First part of 8 mms in contact with agar 2 % containing KCl + $CaSO_4$		31	169	55	208
Second part of 8 mms		19	69	28	98
Third part of 8 mms		9	15	1	58
Fourth part of 8 mms	free part in the light	2	9	11	24
Fifth part of 8 mms		9	2	14	18
		39	95	54	198

Tables 3 and 4 refer to the influence of an exposure of the contactzone of the leaf which is in touch with the agar and the salt. In table 3 the

free part is in the light, in table 4 in the dark. The influence of the illumination is especially clear in the experiments of table 3. Owing to exposure the uptake in the contactzone increases in two experiments from 31 to 169 γ Cl and from 55 to 208 γ Cl i.e. 461 % as an average; the uptake of the free part increases from 39 to 95 γ and from 54 to 198 γ Cl, averagely 305 %. Seeing this free part has to get the salt by transport from the contactzone this zone must have taken in this quantity too, so the uptake by the contactzone has increased in the aggregate from 70 to 264 γ Cl and from 109 to 406 γ averagely 375 %.

TABLE 4.

Influence of an illumination of the contactzone. The free part in the dark. Uptake in γ Cl from agar with 1/100 mol KCl + $CaSO_4$ during 24 hours, 25° C.

		Increase of chlorine in γ.	
	contactzone	dark	light
First part of 8 mms in contact with agar 2 % containing KCl + $CaSO_4$		92	120
Second part		40	136
Third part		26	26
Fourth part	free part in the dark	16	0
Fifth part		12	33
		94	195

The influence of the exposure of the contactzone on the transport to the free part (see table 4) when the free part has been darkened, is 207 %. The effect on the uptake in this case is 141 % and that on the entire uptake 175 %. This experiment corresponds with that of table 3; the effect, however, is less great in this case. We may therefore conclude that exposure of the contactzone increases the uptake of chlorine considerably, both in the contactzone and in the free part of the leaf.

TABLE 5.

Influence of an illumination of the free part of the leaf. The contactzone is in the dark. Uptake in γ Cl from agar with 1/100 mol KCl + $CaSO_4$ during 24 hours, 25° C.

		Increase of chlorine in γ.	
	contactzone	117	120
First part of 8 mms in contact with agar 2 % containing 1/100 mol KCl + $CaSO_4$	in the dark		
		dark	light
Second part of 8 mms		64	61
Third part of 8 mms		17	34
Fourth part of 8 mms	free part	0	24
Fifth part of 8 mms		10	30
		91	149

Table 5 refers to the influence of the exposure of the free part on the uptake by the contactzone, which remains in the dark. When exposed the zones of the free part have a quantity of chlorine of 164 % against 100 % in the dark. This increase probably lies without the limits of error, though the results of these experiments were rather variable. From these experiments it appears:

1. An uptake of chlorine by the contactzone is constantly taking place, it is stronger in the light than in the dark.
2. A transport of chlorine takes place from the contactzone to the free part of the leaf.
3. Both uptake and transport are dependent on the presence of oxygen in the atmosphere.
4. Transport to the free part is greater, when the contactzone is exposed to light.
5. Transport from the unexposed contactzone to the free part of the leaf is probably greater, when this free part of the leaf is exposed to light, than when it is in the dark.

§ 4. *Second series of experiments.*

In these experiments we could avail ourselves of the experience that owing to the cutting of the Vallisneria leaves into pieces of an equal length and width the uptake is checked. From the experiments made on this subject it has appeared that after 24 hours this wound influence has vanished for the greater part, if the leaf lengths are not too short. Therefore the material was always subjected to a 24 hours'preliminary treatment in destilled water in this experimental series.

For an experiment 8 leaves were used of a length of 30 cms, each divided into 4 pieces of 7.5 cms. From these 3 series were formed, each consisting of 8 leaf lengths of 7.5 cms and 3 series consisting of 8 leaf lengths of 2.5 cms, which were used for the determination of the chlorine present, when the experiment was started. Usually these leaflengths were analyzed after a 24 hours'preliminary treatment in destilled water, as during the preliminary treatment the tissue may lose chlorine. Of the 7.5 cm leaflengths the first 2.5 cms, i.e. the contactzone, were placed between agar, while the free part, which had a length of 5 cms, was either placed between moist filter paper or was lying on moist filter paper and covered with a glass plate. As a precaution a little vaseline was put between the contactzone and the free part of the leaf in order to render diffusion along the surface of the leaf impossible. For aeration during the preliminary treatment air free from CO_2 was used. In some experiments air containing CO_2 was used for this purpose. Probably the uptake and the transport are less strong in this case, but this also depends on other circumstances.

On the whole there are 4 different ways of exposure.

1. The leaf lengths entirely exposed.
2. Entirely in the dark.
3. The contact zone exposed and the free part in the dark.
4. The contact zone in the dark and the free part exposed.

Three of these could be compared in one experiment.

Table 6 gives as an example the result of two experiments. For Fig. 1

TABLE 6.

Influence of exposure of the contactzone and of the free part of the leaf on uptake and transport. In each experiment three leaf-series A, B, C with different exposure. Only the first zone is in contact with agar 2 % containing 1/100 mol KCl + $CaSO_4$. The second and third zone are in moist air upon filterpaper. Duration of uptake 24 hours, 25° C. After the uptake the leaflengths were cut in 3 pieces and these were analyzed on Cl content. Pretreatment 24 hours in aerated destilled water in the light.

	A uptake in γ Cl	B uptake in γ Cl	C uptake in γ Cl
First zone of 2.5 cms	light 165	light 199	dark 67
Second zone of 2.5 cms	dark 12	light 64	dark 16
Third zone of 2.5 cms	dark 1	light 49	dark 5
	A uptake in γ Cl	B uptake in γ Cl	C uptake in γ Cl
First zone of 2.5 cms	dark 45	light 116	dark 26
Second zone of 2.5 cms	light 94	light 26	dark 5
Third zone of 2.5 cms	light 53	light —7	dark —14

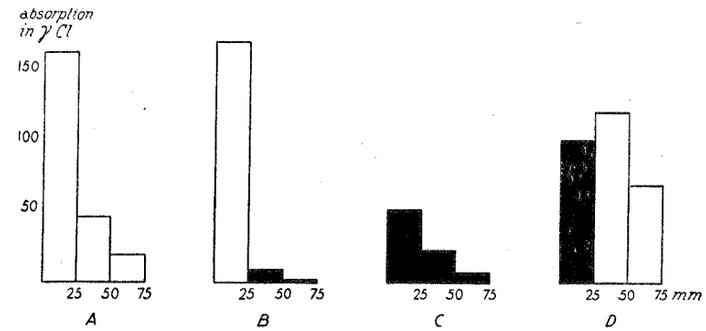

Fig. 1. Influence of exposure to light on uptake and transport of chlorine in leaves of Vallisneria. In *A* the whole leaf is in the light, in *B* only the first zone. In *C* the whole leaf is in the dark, in *D* the first zone in the dark and the free part in the light. The first zone of 25 mms is 24 hours in contact with a solution of 0.01 mol KCl + $CaSO_4$ in 2 % agar. The second and third zone are free in moist air on wet filterpaper. On the ordinate the increase in Cl-content, temp. 25° C. Pretreatment during 24 hours in aerated destilled water in the light.

the averages are used from a greater number of experiments. As discussed in the preceding part, the strength of the uptake is dependent on a great number of factors. As we saw, this obtains for uptake from solution as

well as from agar. Though in these experiments the conditions of exposure, temperature, etc. were kept as constant as possible, yet the differences between the experiments remain great, because the condition of the leaves, when gathered, greatly influences their behaviour and this is a factor which varies from day to day. A 24 hours'preliminary treatment cannot remove these differences.

In the dark, Fig. 1 C, the uptake is slight and so is the transport in every case. The introduction of the preliminary treatment does give much more regular results in this respect than had been obtained in the previous experimental series. The uptake in the light Fig. 1 A is considerably greater than in the dark, especially when during the preliminary treatment there was an exposure to strong light. In one case the transport was also slight in the light. As a rule the 2.5 cm zone bordering on the contact zone gets about 1/3 to 1/2 of the amount of chlorine absorbed in the contact zone. The third 2.5 cm zone gets but little chlorine, sometimes the amount of chlorine does not increase at all here.

If the contact zone is exposed and the free part in the dark, Fig. 1 B, the uptake by the contact zone is fairly normal, but the transport in the darkened free part is very slight.

If the contact zone is in the dark and the free part exposed, Fig. 1 D, a most remarkable phenomenon occurs, which was not found in this way in the first series of experiments (cf. I table 5). The contactzone absorbs more than is the case, when the free part is in the dark and the free part absorbs a great quantity of chlorine, not only the second but also the third zone.

It is noteworthy that in spite of the great variability in the strength of uptake and transport, which again strikes us in these experiments, qualitatively the same phenomena occur in all experiments. To state this it is desirable to study the results of table 7, in which 6 experiments have been

TABLE 7.

Influence of an illumination of the free part of the leaf. The contactzone is in the dark. Uptake from agar with 1/100 mol KCl + $CaSO_4$ during 24 hours. 25° C. Pretreatment of the leaves in destilled water during 24 hours in the light.

First zone of 2.5 cms	contactzone in the dark	147	129	72	88	48	45
Second zone of 2.5 cms	free part in the light	167	140	98	73	91	94
Third zone of 2.5 cms		63	63	46	51	40	53

comprised. Here we regularly find the phenomenon that exposure of the free part causes a strong transport, both to the second and to the third zone. This result deviates a little from what was found in the first experimental series of 1944. In the first experimental series it was only found that on darkening the contactzone the free part takes up more when

exposed than in the dark, but the differences were not very pronounced.

This has given rise to an investigation to find out whether this less pronounced effect in the first experimental series might be connected with the differences in preliminary treatment in the two experimental series. That is why in table 8 some results have been given of a treatment of leaf material subjected to a preliminary treatment in destilled water for periods of various length. In A there was no preliminary treatment, in B it lasted 16 hours, and in C 24 hours. The result of such an experiment, shown in table 8 and fig. 2, is clear. As already discussed before, the absorption is not only checked, but also the transport is strongly affected under the influence of the wounding. Only after a 24 hours'preliminary treatment the

TABLE 8.

Influence of the pretreatment during 0, 16 and 24 hours on the uptake of leaves, treated in the same way as in table 6.

		Pretreatment in dest. water		
		no A	16 hours B	24 hours C
First zone of 2.5 cms	contactzone in the dark	73	122	146
Second zone of 2.5 cms	free part in the light	16	62	157
Third zone of 2.5 cms		— 5	45	104

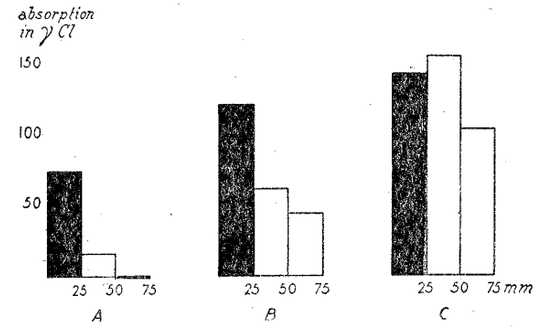

Fig. 2. Influence of pretreatment on the transport of chlorine. Exposure as in fig. 1 D. *A* no pretreatment, *B* 16 hours and *C* 24 hours pretreatment in destilled water. In *C* a strong transport to the free part of the leaves.

result is shown that the free part has increased its amount of chlorine considerably more than the contact zone, which is in direct touch with the medium. It is apparent that after 24 hours pretreatment in water there is an intensive transportation of chlorine from the contactzone to the free part of the leaf.

The results of this second experimental series are therefore a corroboration

of the conclusions drawn from the first experimental series and are a most valuable addition to them.

It appears that the wounding is not only the cause of an inhibition of the absorption, but has also a pronounced influence on the transport. This influence may be considered to contribute to the production of the great differences between the results found in these experiments. *There must be a protoplasmatic factor here which is influenced by the wounding and which is of a dominating influence on the transport.*

§ 5. *Influence of the length of the free part on the absorption by the contactzone.*

Already in the asparagine experiments in 1937 it was observed that in transport experiments made with leaves of a different length, of which a part of a same size acts as a contactzone (i.e. can absorb from the medium), more asparagine is absorbed according as the free part of the leaf is larger. As we saw in the first part of this publication this phenomenon may partly be due to the wounding, which has a stronger influence according as the pieces are shorter and the wound surfaces nearer to each other. For studying this problem it was therefore better not to compare the absorption by a length of 7.5 cms, of which only 2.5 cms are in touch with agar + KCl, with a short length of 2.5 cms which is entirely in touch with the agar, as in this case the wound stimulus might affect the two differently. It is better to compare with an equally large piece of 7.5 cms which is entirely in touch with the agar + KCl and to take for comparison 1/3 part of the quantity absorbed. Table 9 gives the result of such an experiment.

TABLE 9.

Comparison of leaves taking up chlorine only by the contactzone (B and C) with leaves taking up over the whole surface (A). Pretreatment 24 hours in dest. water. Absorption during 24 hours, 25° C, in the light.

	A		B	C
First zone 2.5 cms in agar + KCl	303	in agar + KCl	296	286
Second zone 2.5 cms in agar + KCl	300	between filterpaper	146	128
Third zone 2.5 cms in agar + KCl	300	between filterpaper	17	12
Uptake per zone 1/3 of the leaf	301 γ	total uptake	459γ	426γ

It appears that the two leaflengths B and C, which take up the chlorine in the first zone and transport it to the second and third, have accumulated in their first zone nearly as much chlorine as each third part of leaf A, which is over the whole surface in contact with the medium. Moreover they have transported 163 γ and 140 γ Cl to the second and third zones.

So the total uptake is 459 and 426 γ Cl, while one third part of leaf A gets only 301 γ Cl. This result proves that the transport to the free part of the leaf has not reduced the amount available for the contactzone. This experiment shows that the uptake in the free part of the leaf may be without influence on the accumulation in the contactzone itself.

In various other experiments it was found, however, that part of the transport to the second and third zones may occur at the expense of the accumulation in the contactzone, so that it makes the impression that there is a rivalry among the adjoining zones for the chlorine. The case mentioned first, however, is of essential importance.

In addition to the results already mentioned on page 1240 the following conclusions can be drawn.

1. The transport is strongly affected by the wounds caused by the cutting of the leaves. Though some transport in the first hours is possible a strong transport to the free part of the leaf is only found, when the leaves have been pretreated during 24 hours in destilled water.

2. When during the uptake the contactzone remains in the dark and the free part of the leaf is exposed, there results a strong accumulation in the free part of the leaf. The amount of chlorine present in the second zone is under these circumstances even greater than in the contactzone.

3. Contrary to the rules of a diffusionprocess, a zone lying at some distance from the medium containing the chlorine ions, can take up more chlorine than the zone which is in direct touch with the medium.

4. The amount of chlorine absorbed by the free part of the leaf must have passed the contactzone. In some experiments this resulted in a slighter accumulation in the contactzone, but it also occurred that there was no influence at all. This demonstrates that the uptake in the free part of the leaf is more or less independent of the accumulation in the contactzone.