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Botany. — *On the Mechanism of the Transport of Organic Materials in Plants.* By T. H. VAN DEN HONERT¹). (Communicated by Prof. F. A. F. C. WENT).

(Communicated at the meeting of October 29, 1932.)

In recent years our knowledge about the transport of organic substances in plants has made considerable progress. The valuable researches of MASON and MASKELL (9) not only proved conclusively, that in the cotton plant the transport of sugars takes place in the bark, probably through the sieve-tubes, but also that the direction and velocity of the movement is determined by a difference in concentration. In this respect, the rules governing this process are similar to the laws of diffusion; both are determined by forces which tend to equalize a concentration-difference. However, the rate of transport of sucrose was found to be 20600 to 38450 times greater than could be expected in the case of a transport by diffusion through the sieve-tubes. MASON and MASKELL state this as follows :

¹) By correspondence about the first results of my experiments with Prof. Dr. F. A. F. C. WENT I recently learned from him that, independently, Prof. Dr. F. KÖGL had come to a conception on the transport of his "auxine" (F. KÖGL and A. J. HAAGEN SMIT, these Proceedings Vol. XXXIV N^o. 10, 1931, p. 1411), similar to the ideas developed in this paper.

"The very slow rate of diffusion of sugar molecules in water is of course in a large manner due to the tremendous resistance offered by the water molecules to their movement The sugars appear to move in the sieve-tube under unit gradient at about the same rate as they would diffuse in air if they existed in the gaseous state. The question, therefore, arises whether any mechanism in the sieve-tube can be visualized which would reduce the resistance offered by the medium to diffusion. It is tempting to postulate the existence in the sieve-tubes of some special organization of capillary structure which enormously reduces the resistance to diffusion. We are, however, as yet ignorant as to the possibility of this".

The intention of this paper is to point out that it is indeed possible to visualize such a mechanism.

Further experiments of the same authors gave evidence, that nitrogenous substances (8) and some mineral elements (10) are transported in the same way.

Some remarkable experiments with *Tropaeolum majus* enabled SCHUMACHER (14) to show that of all parts of the phloem the sieve-tubes are the only elements along which nitrogenous organic substances are transported through the leaf-stalk.

These authors have therefore confirmed the most current conceptions on the channels of transport of organic matter, formerly concluded from the results of numerous convincing experiments. As to the mechanism of transport they give us no clue. As a mere diffusion-process entirely fails to explain the rapid translocation of matter, two possible explanations seem thusfar to be left. The first is that originally given by DE VRIES (17), who was of opinion that the protoplasmic streaming is the transporting agent. This opinion is still held by some authors (a.o. CURTIS (2)). However it could not yet conclusively be proved that the protoplasmic streaming is essential in the normal transport through the sieve-tubes.

Another explanation has been given by MÜNCH (11, 12), who developed a new and suggestive theory which drew considerable attention. According to this theory the transport through the sieve-tubes should proceed as a current of solution in the direction from higher to lower turgor pressure. The energy required should be produced in the assimilating leaf cells; like the xylem vessels in the water transport, the sieve-tubes should have only a passive function, serving as semipermeable tubes and being mere channels of transport.

This theory seems to be of high value as a working hypothesis. It will have to be demonstrated whether, under natural conditions, such a rapid movement of water takes place through the sieve-tubes. This may be well within the experimental possibilities.

Some experiments of WEEVERS and WESTENBERG (19) did not confirm some of MÜNCH's experimental results. SCHUMACHER's (14) observation, that nitrogenous substances are rapidly transported out of wilted petals, also seems not to agree with MÜNCH's views.

For a more detailed discussion I refer to comprehensive treatises on this subject (see a.o. WEEVERS (19) ; KRUSEMAN (5)).

As this question seems still far from settled, it may not be superfluous to suggest another possible explanation, which thusfar seems to be overlooked.

When a drop of a liquid, insoluble in water, the molecules of which contain an electropolar group, is placed on a water surface, it will tend to spread rapidly over this surface. This spreading takes place in the direction from lower to higher surface tension and will not stop, until the surface tensions are equalized. This condition will be reached when the layer of the liquid added has become monomolecular. Taking advantage of this phenomenon LANGMUIR (7) in his well-known experiments measured the length and thickness of a number of molecules. The many interesting experiments, since then made on these "two-dimensional" systems have recently been reviewed by HEYMANN (3).

So it is clear that along the surface of water a rapid displacement of matter may take place, originating from surface-active forces. Not only the surface of water against air, but, no doubt, also the interfacial surface between water and another liquid may constitute such a channel of transport.

In order to demonstrate this in a clearly visible way an apparatus was constructed, which, after several trials, is shown in its final shape in figure 1.

Two small glass bulbs *a* and *b* with a volume of about 7.5 cc each are

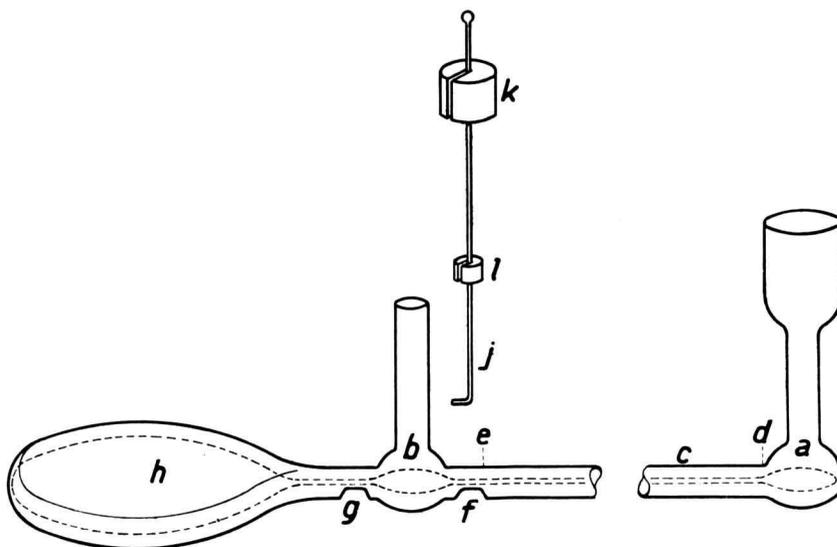


Fig. 1.

connected with a tube *c*. This tube has an inner diameter of 1 cm. The distance between the centres of the bulbs is 1 m. Bulb *a* is provided with

a funnel. To the other side of bulb *b* another similar tube is fused, connecting it with the receptacle *h*. This receptacle is of a flat elliptical shape, with the dimensions of $1\frac{1}{2} \times 10 \times 8\frac{1}{2}$ cm.

At both sides of bulb *b* the bottom of the glass tubes *f* and *g* has been elevated over a distance of 1 cm, in such a way that the inner side of the glass bottom nearly reaches the middle of the tubes.

In bulb *b* a small stirrer *j* can be inserted consisting of a tiny glass rod, 1 mm thick and bent at right angles at the lower end. It is inserted in two pierced corks *k* and *l*, which are provided with a slit. Cork *k* is to be fixed in the clamp of a stative, whilst the small cork *l* is inserted in *b*. By rotating between the fingers the head of *l* contents of *b* may be stirred without shaking the apparatus.

One liter of distilled water was slightly acidulated with hydrochloric acid and 25 cc of a 0.04% solution of the indicator chlorphenol red (Dichlorophenolsulfonphthalein) was added.

After boiling it to expel the dissolved carbon dioxide, traces of *KOH* were added to give it a *pH* of 5.8. At this *pH* the indicator shows an intermediate color and a shifting of the *pH* in either direction may be easily observed.

The apparatus was half-way filled with a measured quantity of this solution and subsequently filled with pure ether saturated with water, precautions being taken to leave no air-bubbles inside. By gentle shaking during several minutes the water was also saturated with ether. Subsequently the apparatus was exactly leveled horizontally, so that the interface between ether and water divided the apparatus into an upper and a lower half, as indicated by the dotted line in the figure, the water only just covering the elevations *f* and *g*.

In 25 cc of a 0.2 normal solution of potassium hydroxide 0.1 g oleic acid was dissolved. When a small quantity of this oleate solution containing excess of *KOH* is put into bulb *a* the orange-red color of the bulb is converted into purple. This color change is not confined, however, to the contents of bulb *a*, but immediately begins to spread along the water-ether interface. Seen in side-view, it has the shape of a sharp wedge, moving along the interface at first rapidly, afterwards more slowly. Evidently a rapid displacement of potassium oleate takes place along the interface. When the oleate comes into contact with the trace of *HCl* in the water it is converted into oleic acid, making room for a new spreading of oleate and resulting in a shifting of the *pH* towards a more alkaline reaction.

It is, in this case, not only a movement of a "two-dimensional" film. A layer of water (and, of course, of ether) is dragged along with the interface, causing a distinctly observable compensating stream of water along the bottom of the tube in opposite direction. So the movement is in fact a displacement of a certain volume of solution. The neutralisation reaction therefore will be due probably for the greater part to the excess of hydroxyde contained in this solution. However, since the oleate, according

to GIBBS' rule (KRUYT (5), p. 18), accumulates in the interface, the share of hydroxyde and oleate in the transport of alcali is hardly to estimate.

If the receptacle *h* were omitted and the apparatus ended at *g*, the inevitable contaminations of the ether-water interface would accumulate in *b* and seriously interfere with the experiment. If they are allowed to escape into *h*, the spreading substance may enter into bulb *b* unhampered.

After adding a certain amount of the oleate solution to bulb *a*, one may observe the velocity with which the purple color spreads along the interface, thus stating that measurable amounts of alcali are transported over a certain distance within a certain time. In this way one obtains information about the velocity of transport but not about the quantities transported.

Therefore a measured quantity of 0.01 normal hydrochloric acid was added to bulb *b* and the time, necessary for the neutralization of the acid, was measured. In this manner the quantity transported within a certain time could be approximated.

The procedure of the experiments was as follows :

A thin graduated pipette filled with 0.01 normal hydrochloric acid was carefully introduced into bulb *b*, so that the point reached the bottom and a quantity (0.6 or 1.2 cc) of *HCl* was slowly added. Then the pipette was gently removed and the stirrer put in its place, so that its lower end reached beneath the water-ether level. The acid added proved to remain for the greater part unmixed in the lower part of the bulb *b*, between the elevations *f* and *g*.

Now 1 cc of the oleate solution was added to the contents of bulb *a* by running it along the inner side of the funnel. The addition took about four seconds. From the moment of the first contact of the oleate solution with the interface the time was measured, in which the purple color covered a distance of 25, 50, 75 and 95 cm from point *d*. Then the stirrer was put into action and all alcali transported into bulb *b* was immediately mixed. The time was observed at which the color change of the contents of *b* was completed after the moment that the purple color had reached the 95 cm point at *e*.

As a blank determinations were also made without addition of hydrochloric acid.

The determinations are, of course, rather rough. Several sources of error are not taken into account. After addition of the oleate solution it is diluted to an unknown extent by the indicator solution. Besides, the depression of the interface tension causes a slight lowering of the level and a little shifting of the water layer from *a* towards *b*. By this movement also traces of *HCl* may escape into *h*, so that the orange-red color of the indicator locally changes into yellow. Also the higher specific weight of the oleate solution compared to the ether-saturated water may introduce a slight error. However, these errors may hardly have exerted a serious influence upon the results, especially since these observations only intended to determine the order of magnitude of this mode of transport.

The results of several determinations are given in the next table.

HCl 0,01 N added to <i>b</i>	Distance 25 cm.	Covered 50 cm.	In seconds 75 cm.	95 cm.	Contents of <i>b</i> neutralized	Average
None	9	65	175	293	42	40
"	8	60	160	280	40	
"	8	85	232	398	37	
0,6 cc	9	85	220	368	198	186
idem	9	85	235	403	182	
"	9	80	204	353	183	
1,2 cc	9	71	205	358	232	239
idem	10	100	265	445	245	
"	9	100	313	520	240	

It is evident that the spreading-velocity is very variable. However, the time wanted to neutralize the contents of bulb *b* seemed to be fairly constant; 0.6 cc *HCl* 0.01 *N* were neutralized in $186 - 40 = 146$ seconds; 1.2 cc in $239 - 40 = 199$ seconds. Since it took 146 seconds to neutralize the first 0.6 cc, the next 0.6 cc of this 1.2 cc are evidently neutralized in $199 - 146 = 53$ seconds. This means a rate of transport of 0.006 milliequivalents in this time.

We may now visualize, in the same way as MASON and MASKELL did, that this transport is effected by a mere diffusion of *KOH* through the water layer and roughly estimate the "diffusion constant" belonging to this hypothetical process. We may assume that at this time of the experiment the oleate solution in *a* will have been considerably diluted, so that for instance the concentration of *KOH* will have become 0.02 milliequivalents per cc. The water layer connecting the bulbs is 97 cm long and (neglecting the constriction at *f*) has a cross-section of about 0.3 cm². Furthermore, we assume that there is a linear and constant concentration gradient from *a* to *b* during the 53 seconds concerned.

According to FICK'S law we may now compute the "diffusion constant" as follows:

$$\frac{0,006}{53} \cdot \frac{1}{0,3} \cdot \frac{97}{0,02} = 1,83$$

The real diffusion constant of *KOH* in diluted solution at 18° C is 2.2×10^{-5} (International Critical Tables (4)). At the temperature at which the experiments were made (about 27° C) this constant may be

2.7×10^{-5} . The rate of transport observed is therefore 68000 times greater than could be expected.

If we assume that the greater part of the potassium is transported in the form of oleate, a still greater disproportionality should result from the computation. In any case, the velocity of the process is of the same order of magnitude as that found by MASON and MASKELL in the transport of carbohydrates.

Such a comparison of the velocities of the spreading and of the diffusion, however, is not correct, since the processes depend upon different factors in a different way. The spreading velocity will a.o. be dependent upon lowering of the interface tension by the interface-active substance; upon the viscosity of both fluids limiting the interfacial surface, and upon the diameter of the tube. The viscous resistance will increase with decreasing distance of the interface to the wall of the tube, so in very narrow tubes the disproportionality will not be so great. On the other hand, in narrower tubes the ratio of interface to cross section will increase, which will have an effect in opposite direction.

When we omit the ether layer from the experiment and the spreading proceeds along a water-air surface there is less viscous resistance and more lowering of the surface tension. Indeed, under these circumstances the purple color covers the distance of 95 cm in 18—20 seconds. In this case this spreading is preceded by a visible depression of the surface tension, running through the tube like a wave and reaching point e in only 5 seconds. In this short time traces of oleate must have been transported along this distance.

In one respect this mode of transport is similar to diffusion. As the lowering of the interface tension is dependent upon the concentration of the interface-active substance, the rate of transport will be a function of the concentration gradient, though perhaps not a linear one. In this regard as well as in regard to the order of magnitude, this is in accordance with MASON and MASKELL's experiments.

We may now look for an interface which might constitute a channel of transport in the sieve-tubes. It seems to me that the interface boundary between protoplasm and vacuole should meet the requirements. In any case it is an interface between two immiscible liquid phases and any substance lowering its tension would tend to spread along this interface until concentration-differences are equalized. This should of course be accompanied by protoplasmic streaming which could, however, be the consequence instead of the agent of the transport. The primary cause of the transport would be only the concentration-difference of the substance to be transported.

The observed influence of narcotics upon the rate of transport of carbohydrates seems to agree with the above developed conception rather than with the theory of MÜNCH. Narcotics are all surface-active sub-

stances; so they probably accumulate in the interface and displace the substances normally to be transported.

I do not venture to pretend that, along this line, a satisfactory explanation will be at once found. Several difficulties certainly will be met with. Sugars, for instance, show a negative surface activity in the surface water-air. In accordance with GIBBS' rule they are negatively adsorbed by heat-denatured serum-albumine from their aqueous solution (BERCZELLER (1)). However, they are positively absorbed by a coal-suspension. This seems to indicate that the adsorption depends upon the nature of the interface and that the interface tension in the coal-water interface is lowered by sugars (RONA and MICHAELIS (13), VASATKO (16)). The same might be the case in other interfaces. It might also be possible that sugars were temporarily transferred into some interface-active form, in the same way as potassium is positively interface-active in the form of the oleate but negatively interface-active in the form of the hydroxide.

The relatively slow displacement of rotating protoplasm may be felt as an argument against this conception.

I will not go further than to indicate some new questions which are brought out by these experiments. For the present, a relation between the spreading of a substance along a liquid surface and the transport of organic substances through the phloem may seem to be rather theoretical. A more thorough study may prove whether the idea, indicated in this paper, is erroneous or, perhaps, suitable as a working hypothesis.

Proefstation voor de Java-Suikerindustrie.

Pasuruan, September 1932.

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