BOTANY

ION-SECRETION INTO THE XYLEM AND OSMOTIC REGULATION OF EXUDATION

BY

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The phenomenon of exudation in plants has been the subject of many investigations. We shall confine ourselves here to those exudations of herbaceous plants where the sap contains exclusively salts. Some authors are of the opinion that the root acts as an osmometer. In consequence of the difference in osmotic concentration between exudated sap and outer solution, the sap in the xylemvessels is diluted. For the continuation of the exudation osmotically active substance must be secreted into the vessels. (EWART, ATKINS, PRIESTLY, SABININ, CRAFTS and BROYER, HOAGLAND). VAN OVERBEEK presumes that along with the osmotical action there is an active water secretion into the xylem. EATON discards this view and surmises that the mannitol used for compensation of the exudation pressure penetrates into the root-tissue. As a consequence too high a value will be found for the compensating osmotic value.

LUNDEGÅRDH described the exudation phenomena in a large number of papers. At first (1943) he considered these as an osmotic process in combination with an active anionsecretion into the xylemvessels. The possibility of polar watertransport was considered, too. In 1945 he assumed a secretion of a salt-solution by parenchymatic cells in the stele. In 1949 he stated that the secretion of cations into the xylemvessels was regulated by a glycolytical system. The anion-respiration, in his opinion, had only influence on the transport of anions in the protoplasm to the secreting cells.

The immediate cause of our investigation was the wish to examine the problem of active water-secretion more closely. Only a short outline of the results obtained will be given in this preliminary note.

Experiments.

After the tomatoplants had grown from ten to fifteen weeks, they were cut the evening before the experiment was carried out. The following day, during the experiment, either a tube with narrow bore was placed on the stump to collect the sap, or a potometer tube for rate determinations. The roots were placed in a large funnel in order to facilitate the changing of the solution. Especially the influence of changes in the osmotic value of the medium on the exudation process was investigated.

The different osmotic values were mostly obtained by adding different amounts of mannitol either to a Hoagland-solution or to water. In this way two kinds of experiments were made.

The first kind is illustrated by fig. 1. A root system is placed alternately on a Hoagland-solution and a similar solution of which the osmotic

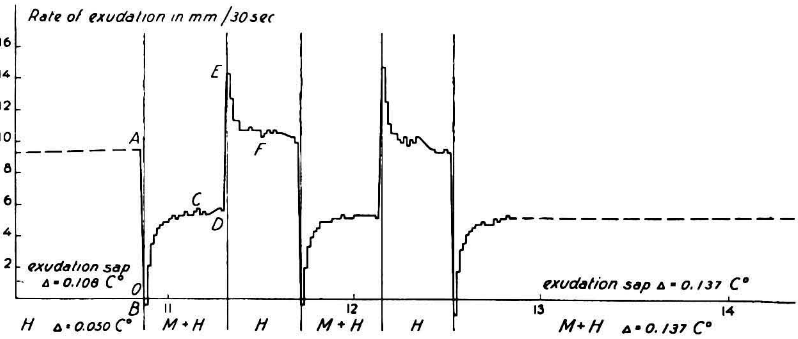

Fig. 1. Influence of changing the osmotic value of the medium on the exudation. The tomato plant is continuously on a Hoagland solution (H). The osmotic value is increased by adding mannitol. (M + H).

value was increased by the addition of mannitol. It is apparent that in changing the solutions a sudden strong change in the exudation occurs, followed by a more gradual change in the reverse direction. This slowly leads to a new equilibrium, which is attained after some 10 minutes. It is remarkable that the sudden decreases (fig. 1 *A B*) have the same value as the sudden increases of the exudation (fig. 1 *D E*).

During this experiment exudation sap was collected three times. Of these and of the outer solutions used the freezing point was determined (fig. 1). It is obvious that in placing a root system on a medium with a higher osmotic value the exudation decreases whereas the osmotic value of the exuded sap increases. The osmotic value is also a consequence of the salt-secretion, the strength of which can be calculated by multiplying the rate of the exudation by the osmotic value of the sap.

Here we start from the supposition that the concentration of the sap in the regions of the root system where these processes take place, equals the concentration of the exuded sap. In this experiment the salt secretion on the stronger osmotic medium was found to be smaller too.

Other investigators have found an identical course of the exudation. They ascribe the slow change in the exudation that appears after the sudden decrease or increase in changing the solution, to a permeation of the substance used. (Sabinin 1925, Eaton 1943). To test this supposition, an experiment was made as illustrated in fig. 1 but this time by increasing the osmotic value not only with mannitol but also with raffinose and thiourea. Though it may be surmised that these substances will permeate in a different degree, no distinct differences were found.

The osmotic value of the medium was also altered in the experiments of the second kind. But in contrast with the first series we did not wait for the attainment of a new equilibrium. Thus it was possible to trace out, in the morning, as well as in the afternoon, the sudden effect of five solutions of different strength on the exudation. The result of such an experiment is represented in fig. 2. On the abscissa is given the osmotic

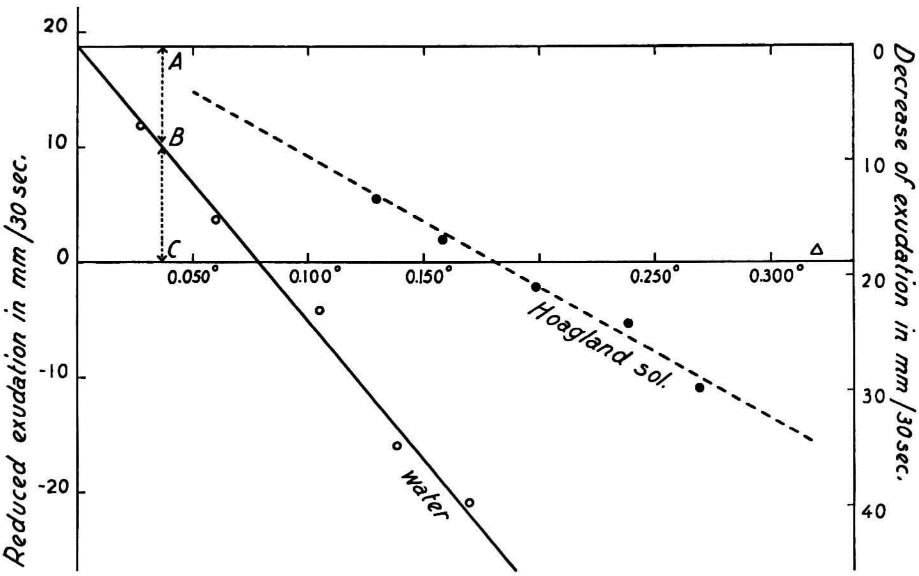

Fig. 2. Relation between osmotic value of the medium and the reduced exudation (left ordinate).

value of the mannitol solutions, on the ordinate the sudden decrease (AB) of the exudation resp. the reduced exudation (BC). The regression lines were computed. The figure shows a distinct rectilinear proportionality between the change of the exudation resp. the reduced exudation and the osmotic value of the medium. As well as for a root system on water, the regression line for the relation between reduced exudation and osmotic value of the medium is drawn for a root system on Hoagland solution (fig. 2, dotted line). The course of the regression line for the plant on water is steeper than that for the plant on Hoagland solution. This means that changes in the osmotic value of the medium have a greater effect with a plant on water: the resistance for the transport of water is smaller.

The point of intersection of the regression lines with the abscissa gives the osmotic value of the medium which brings the exudation momentarily to a standstill (compensating osmotic value). In comparing these osmotic values with those of the exuded sap we encounter the difficulty that these values vary greatly during the day. It seems appropriate to confine ourselves to those experiments in which this periodicity is smallest, and which are therefore better to compare. We obtained the following results.

Osmotic value of the exuded sap	Compensating osmotic value	Difference
0.060	0.079	0.019
0.039	0.077	0.038
0.137	0.180	0.043
0.138	0.156	0.018
	Average difference 27 %	

As a result we find that the osmotic value of the exuded sap is about 0.5 atm. lower than theoretically from an osmotic standpoint could be presumed. It may be stressed that the difference is very variable and more than one atm. smaller than that found by VAN OVERBEEK.

Theory of the exudation.

We shall start our theoretical considerations from the osmotic view and will examine whether our experimental results are in accordance with this theory. So our working hypothesis is that the root system functions as an osmometer. We have according to SABININ (1925):

$$b = k(O_b - O_m) \tag{1}$$

when b represents the rate of exudation, O_b the osmotic value of the exudate, O_m that of the medium and k a factor of proportionality.

From this formula it follows first of all that the exudation stops when we make the osmotic value of the medium equal to that of the sap. We found, however, that the osmotic value of the sap was on the average 27 % lower than the compensating osmotic value of the medium.

It should be borne in mind that the experimental errors are rather great ones, and that there is a great variety in the differences.

Moreover, we may expect according to (1) that in changing the osmotic value of the medium O_{m1} to O_{m2} the exudation b_1 will change suddenly and in proportion to the osmotic value of the medium to b_2 according to.

$$b_2 - b_1 = k\,(O_{m1} - O_{m2}) \tag{2}$$

This actually happens (fig. 2) and in accordance with KRAMER we consider this to be a strong proof for the osmotic regulation between sap and medium.

However, the changing of the osmotic values of the medium will have still other consequences. As the osmotic value of the sap O_b results from the salt secretion S and the exudation b, the following relation obtains between these three factors.

$$S = b\,O_b \tag{3}$$

It is obvious that as soon as the exudation is suddenly altered (2), while the salt secretion continues, the osmotic value of the sap changes too (3). A period begins in which the exudation gradually alters in a direction counter to the preceding sudden change till a new level of equilibrium is reached. For these states of equilibrium we may derive from (1) and (3) that

$$(4) \qquad b = -\tfrac{1}{2}\, kO_m + \sqrt{(\tfrac{1}{2}\, kO_m)^2 + k\, S}$$

It is apparent that according to the osmotic theory the exudation is dependent on the salt secretion, the osmotic value of the medium and the factor k, which is inversely proportional to the resistance of the root system for water-transport.

In order to form an opinion as to whether our results were in accordance with this formula we evaluated the salt secretion which pertains to the exudations at the state of equilibrium mentioned in fig. 1. The factor k can be derived with the aid of (2), the osmotic values of the medium are known. We can calculate the theoretical osmotic value of the exudation sap from the salt secretion and compare this value with the value found. The result is:

Medium	saltsecretion	osmotic value of the exuded sap calculated	osmotic value of the exuded sap determined	difference
Hoagland	1.35	0.133	0.079	27 %
Mannitol + Hoagland	0.97	0.181	0.137	24 %

It is apparent that also for exudations in the state of equilibrium too low a value has been found for the osmotic pressure of the exudation sap and that the difference is of the same order as above.

We have mentioned already that there is, between the sudden decrease of the exudation and the more or less constant exudation at the state of equilibrium, a period of gradual increase. We can now by means of (1) and (3) arrange a formula for the relation between exudation and time. We have to start from the supposition that the salt secretion is constant during this period and we assume that as well as the water absorption it is limited to a region of the root, here designated as the active volume V.

The formula is as follows:

$$(5) \qquad b = \tfrac{1}{2}\, kO_m + \sqrt{(\tfrac{1}{2}\, kO_m)^2 + k\, S} \cdot \mathrm{tgh} \left(\frac{\sqrt{(\tfrac{1}{2}kO_m)^2 + k\, S}}{V} \cdot t - C \right)$$

$C =$ integrationconstant

A part of the experimental data on the exudation from fig. 1 has been plotted in figure 3 together with a curve giving the values of exudation as calculated according to (5). There is apparently an adequate agreement.

Summing up we can say that the course of the exudation in our experiments can satisfactorily be accounted for. The level of the exudation is, however, higher than can be expected on the ground of the osmotic value of the exuded sap. This can be explained by assuming that the sap, during its transport to the stump, loses part of its salts or takes up extra water by osmosis. Either by one or by both processes a dilution effect results. In that way it is even theoretically thinkable that the osmotic value of the sap that is exuded, is lower than that of the medium. The difference may also be explained by accepting the view that there is

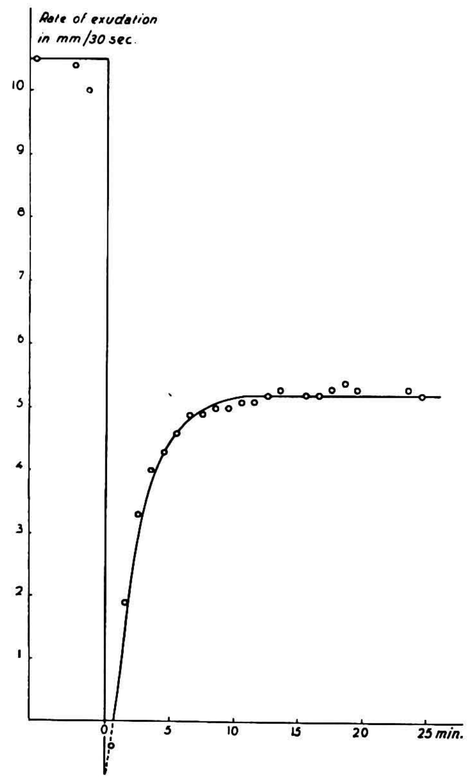

Fig. 3. Comparison between experimental data (○○○) and the calculated course of the exudation during the period in which the exudation increases by the continuation of the salt secretion.

still another process that transports water in a non-osmotic way. This does not mean the acceptance of an active water secretion. It seems possible that this process is bound in one way or another to the mechanism of the salt secretion, e.g. in the way supposed by LUNDEGÅRDH. Considering the importance of the osmotic regulation it does not seem very probable that such a process could produce a great difference between the osmotic values of the sap and the medium. Actually we found as largest difference that the freezing point of the exuded sap was only 0.009° lower than that of the medium. Further research, especially on the relation of the salt secretion to the exudation, will have to decide which of the suppositions is the right one. So it can not yet be stated whether the exudation process is partly caused by a non-osmotic water transport; anyhow, it is certain that the osmotic regulation is of outstanding importance.

Discussion.

We have found that small changes in the osmotic value of the medium produce distinct changes in the exudation. This indicates a low resistance of the root system for water transport as mentioned also by other investigators. (KRAMER 1945, 1949; LUNDEGÅRDH 1945). Moreover, it points to an immediate osmotic regulation of the exudation as can be expected according to the formula of SABININ (1925): $b = k(O_b - O_m)$. LUNDEGÅRDH, in his latest paper, attaching only a very small importance to the osmotic value of the exudation sap, finds a relation between the square root of the osmotic value of the medium and the strength of the exudation. On this ground he gives the following equation

$$b = k\,(\sqrt{P_e} - \sqrt{P_m}).$$

The relation we found between changes in the osmotic value of the medium and of the exudation appears to be rectilinear, which is in close agreement with the formula of SABININ, but contrary to that of LUNDEGÅRDH.

We have to consider that we investigated momentary effects while LUNDEGÅRDH determined the exudation during longer periods. For this purpose we may make use of formula (4) giving the relation between the exudation in the state of equilibrium and the osmotic value of the medium. Calculation with this formula (4) shows us that between definite limits the exudations actually are proportional to the square root of the osmotic value. In using this formula we neglected a possible influence of the osmotic value of the medium on salt secretion. This result can explain the proportionality found by LUNDEGÅRDH with $\sqrt{O_m}$.

Just as VAN OVERBEEK we determined the compensating osmotic value of the medium and compared it with the osmotic value of the exuded sap. We had the same result and found the last one always higher than the first though the differences were much smaller. This can be explained by the difference in method. VAN OVERBEEK added the mannitol slowly by drops until the exudation was stopped for five minutes. Now we know that in this time the osmotic value of the sap will be increased. He obtained therefore too high a figure for the compensating osmotic value. Moreover too low an osmotic value of the sap exuded from the stump after being replaced in water, will be found.

EATON mentions also the gradually increasing exudation after the momentary sudden decrease. He explains it by the penetration of the osmotic active substance from the medium into the root system. By means of substances which permeate in a different degree into the cell we could ascertain that permeation phenomena were out of the question. Moreover we concluded that it is superfluous to assume a separate process for this phenomenon. The salt secretion being the active force of the exudation, a sudden check of the exudation must be followed by a gradual increase, since the osmotic value of the exudation sap increases.

Summary.

The influence of a change in the osmotic value of the medium on the exudation of tomato-plants was investigated. An enhancement of this value is followed by a sudden decrease of the exudation passing into a gradual increase. A new but lower level is attained after about 10 minutes; the osmotic value of the sap is than higher. Lowering of the osmotic value of the medium gives opposite results. As it makes no difference whether mannitol raffinose or thiourea are supplied to the medium, this gradual change cannot be the result of permeation, as was supposed by SABININ and EATON. This phenomenon must be ascribed to the continuous salt secretion into the xylemvessels.

We found the sudden decrease of the exudation, caused by placing the plants on higher concentrated external solutions, proportional to the osmotic values of the medium. This agrees with the osmotic theory but is contrary to the theory and formula of LUNDEGÅRDH (1945, 1949). For prolonged experiments another formula could be derived, by which the conflicting results may be partly explained.

By means of the experiments just mentioned the osmotic value of the medium that momentarily stops the exudation could be determined. Comparing this value with that of the sap it appears that for the latter always too low a value is found. The differences are very variable, but always much lower than the differences found by VAN OVERBEEK, which could be ascribed to our improved method of determination.

The only deviations which we found in testing our theory, were the values of the exuded sap, which were too low. The course of the exudation found in our experiments was in perfect agreement with our theory, which indicated that the osmotic component of the exudation was of paramount importance for this process.

The too low values found for the exudation sap can be ascribed either to a difference between the exuded sap and the sap present in the active region of the root, caused by saltabsorption and water-dilution, or to a non osmotic water-transport. Whether this latter process really exists will have to be decided by further research, especially into the relation between salt secretion and exudation.

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