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THE INFLUENCE OF SALTS ON THE EXUDATION OF TOMATO PLANTS

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CONTENTS

I. INTRODUCTION 445
II. MATERIAL AND METHODS 449
III. DESCRIPTION AND DISCUSSION OF THE EXPERIMENTS 453
§ 1. *Influence of changes in the osmotic value and the salt concentration of the medium on the exudation* 453
§ 2. *Relation between salt condition of the plant and salt secretion into the vessels* 466
§ 3. *Influence of the composition of the medium on the exudation* 467
a. Relation between composition of the medium and the rate of exudation. 467
b. Concentration and composition of the exudation sap in relation to the composition of the medium 470
c. Influence of the pH of the outer solution on the exudation 478
§ 4. *Stimulation of the exudation under the influence of sugar* 480
§ 5. *Changes in the salt secretion as a result of alterations in the osmotic value of the medium* 485
§ 6. *The influence of inhibitors on the exudation*. 487
a. Potassium cyanide 487
b. 2,4-Dinitrophenol 491
c. Sodium arsenate. 492
d. Sodium fluoride 494
e. Iodo acetic acid 496
f. Phenylurethane 497
§ 7. *Periodicity* 500
IV. GENERAL DISCUSSION 506
V. SUMMARY. 516

I. INTRODUCTION

In 1874 it had already been pointed out by SACHS that two types of exudation can be distinguished, i.e. the exudation which must be attributed to the occurrence of a local pressure in the stem, and the exudation caused by a process localized in the roots. The first phe-

nomenon is usually shown by trees. The second, although recorded also from trees, has been investigated mainly on herbaceous plants. We shall confine ourselves here to the exudation of decapitated plants, which is caused by the development of a root pressure. Many theories have been advanced on the causes of the development of root pressure. Some of them will be mentioned here. For a comprehensive review of older literature the readers are referred to HEYL's paper (1930); more recent literature has been summarized by KRAMER (1949) and ARNOLD (1952).

By many authors the water transport in exudation has been considered as a movement along gradients of increasing osmotic pressure. PRIESTLEY (1920) thought that cells adjacent to the vessels would have the highest concentration of osmotically active substances, so that water would be sucked in. As the cells between the endodermis and the vessels cannot expand, water would be pressed into the vessels. In this case it must be supposed, that the cells are more permeable to water on the inner than on the outer sides, a possibility which had already been mentioned by PFEFFER.

V. H. BLACKMAN (1921), however, pointed out, that it is not necessary to assume an osmotic gradient in the root tissue. According to his opinion the osmotic value of the cells should be of no importance, if only there is a difference between the osmotic value of the sap in the vessels and that of the medium. This view has been elaborated by SABININ (1925). He considered the action of the root as that of an osmometer. According to this author the rate of exudation should depend on the difference between the osmotic value of the medium and that of the sap in the xylem vessels, so that $b = k\,(P_x - P_e)$, b being the rate of exudation, P_x the osmotic value of the sap, P_e the osmotic value of the medium and k a constant. The root tissue must act as a semipermeable membrane. The values of P_x calculated from this formula agreed with the osmotic value of the sap.

RENNER (1915, 1929) stated like SABININ that changes in the osmotic value of the medium influenced the rate of exudation, in agreement with the osmometer theory. This was also confirmed by KÖHNLEIN (1930). EATON (1943) established in cottonplants a rectilinear relation between the rate of exudation and the difference between the osmotic value of the sap and that of the medium thus supporting SABININ's idea. KRAMER (1941) found that the exudation could be converted within one minute into absorption by enhancing the concentration of the outer solution. The reverse held true also. According to his opinion this can be explained only if the roots act as an osmometer.

Although these facts indicate the importance of an osmotic process for the transport of water, this cannot explain the whole process of exudation, as BLACKMAN has emphasized, since necessarily a certain concentration difference must somehow be maintained between the solution in the vessels and the external solution. According to PRIESTLEY the content of cells differentiating into xylem vessels would provide a supply of osmotically active material (mainly sugar). It has been proved however, that exudation sap generally contains little or

no sugar at all, but mainly salts. CRAFTS and BROYER (1938, c.f. also CRAFTS, CURRIER, and STOCKING 1949) supposed salts to diffuse through the symplast to the stele. Owing to the lower metabolic status (a result of the lower oxygen pressure) the cells in the stele cannot retain the same salt concentration as the cells in the outer layers of the root tissue, so that leakage of solutes to the vessels must take place causing a suction of water along the cell walls.

HOAGLAND and BROYER (1942; HOAGLAND, 1948) attributed root pressure to accumulation of salts and salt transport to the vessels. Since these processes are controlled by metabolism, the influence of oxygen and narcotics on the exudation would be explained. Nevertheless they thought it very probable that these phenomena could not be entirely explained in this way, especially on account of the influence of auxin on the exudation process, which had been found by SKOOG, BROYER and GROSSENBACHER (1938).

When investigating the exudation of tomato plants ARISZ, HELDER and VAN NIE (1951, c.f. VAN NIE, HELDER and ARISZ, 1950) started from the supposition that the exudation would consist of two processes: an active transport of salts to the xylem vessels and an osmotic suction of water by the xylem sap. If the osmotic value of the medium was altered, the rate of exudation showed a sudden change. A rectilinear relation could be demonstrated between the concentration of the medium and the sudden decrease of the rate of exudation caused by it. This provides an important support to SABININ's theory. Like this author they came to the conclusion that the rate of exudation was proportional to the difference between the osmotic value of the sap and that of the external solution: $b = k\,(O_b - O_m)$. The factor of proportionality, k, has to be considered as a measure of water conductivity of the root (b is the rate of exudation as in the formula of SABININ, and O_b and O_m are the osmotic value of the sap and that of the medium). In this case the rate of exudation would be controlled by two internal factors, k and the osmotic value of the sap. The latter depends on the secretion of salt into the vessels and on the transport of water, that is on k.

Like SABININ, RENNER and EATON, ARISZ, HELDER and VAN NIE found that if the osmotic value of the medium was enhanced, the sudden decrease of the rate of exudation was followed by a more gradual increase till a new equilibrium was attained. They pointed out that this could be explained by means of the theory mentioned above without assuming the permeation of substances from the medium. The fact is, that when the transport of water has been decreased by enhancement of the osmotic value of the medium, the sap must become more concentrated if the secretion of salts into the vessels continues at the same rate as before. This means an increase of the osmotic value of the sap and thus as the discrepancy with the osmotic value of the medium becomes greater, an increase of the rate of exudation also. Conversely a dilution of the sap, and a decrease of the rate of exudation are to be expected when the osmotic value of the medium is lowered. These changes of the osmotic value of the sap have actually been found.

The exudation phenomena, which were observed, could be explained for the greater part by means of the theory with which they started but there appeared to be one exception. The osmotic value of the sap was in most cases lower than would be expected. ARISZ, HELDER and VAN NIE did not consider this a reason to assume the occurrence of an active water transport as well as the active transport of salts. According to their opinion the transport of water and salts cannot be separated. In addition they mentioned the possibility that the osmotic value of the exudation sap is different from that of the sap in the vessels of the roots.

In the papers which have been discussed above, the exudation was considered as the result of a difference of osmotic value between the sap in the vessels and the medium. Now we must mention the view of some authors who hold another opinion.

SPEIDEL (1939) considered the salt transport of no importance. His idea concurs more or less with that of PRIESTLEY: the cells surrounding the vessels should have the highest osmotic value and attract water. By enzymatic processes, osmotically active substances are broken down continually and in consequence of this the osmotic value decreases, so that water which had been bound osmotically is pressed into the vessels.

VAN OVERBEEK (1942) distinguished an osmotic as well as an active water transport. He found, that the concentration of the external solution that would fully inhibit the exudation of tomato plants, was always much higher than the osmotic value of the exudation sap. This would indicate the presence of an active component. Only the latter was sensitive to potassiumcyanide. ARISZ, HELDER and VAN NIE however offered some criticism on VAN OVERBEEK's method of determining the root pressure. BROYER (1951) following VAN OVERBEEK's method did not succeed in obtaining wholly satisfactory results.

ARNOLD (1952) concluded from facts in the literature on the uptake and secretion of water and salts that the transport of water should take place entirely independently from the transport of salts. The protoplasm of the cells would, according to his opinion, take up water both osmotically and electroosmotically. The intensive transport of water through the endodermis, however, was considered by him to be an active process, which could also take place against a concentration gradient.

The theory of LUNDEGÅRDH (1945, 1949, 1950) holds more or less a position of its own. This author considered the transport of salts of importance for the exudation of wheat plants, but rejected his former view that the transport of water would be caused by the difference between the osmotic value of the sap and that of the external solution (1940, 1943). According to his opinion anion respiration, pumping salts into the cells, should be the driving force of the exudation process: "Anion respiration and a comparatively high exchange permeability together build up the steady state of salts, viz. the concentration level of salts in the root tissue, the former representing the up-hill reaction, the latter the down-hill reaction". The exudation

of salts from the cells is to be considered as a passive process. When salt is given off from the cells, there is a change in their osmotic value. The turgor is decreased and besides salt, water is expressed. This salt solution is exuded by the vascular epithelium, the cells adjacent to the xylem vessels.

The movement of water is impeded by an increase of the osmotic value of the medium. This causes the exudation of a smaller amount of the salt solution, but the concentration of the solution is not changed. According to LUNDEGÅRDH's view, besides this solution, water without salt is given off to the vessels. This is water, which has been released when osmotically active substances are converted into inactive substances in synthetic processes. This so-called extra water secretion is inhibited by sodium fluoride, which proves that the process is connected with glycolysis. In the third place water together with carbondioxide, produced in respiration, can be transferred to the vessels. Afterwards the carbondioxide can escape from the vessels.

The experiments, which are described below, form a continuation of the investigation of ARISZ, HELDER and VAN NIE.

The osmometer theory has been used as a working hypothesis; that is we have supposed that the exudation process is based on a transport of salts to the vessels causing a concentration difference between the xylem sap and the medium, which results in an osmotic transport of water.

Because of the possibility of a close relation between water and salt transport (ARISZ, HELDER and VAN NIE; LUNDEGÅRDH) it seemed logical to try in the first place to obtain a better understanding of the process of salt secretion. To this end the influence of several factors on the process has been investigated. The effect of changes in the salt concentration of the outer solution proved to be very important. This can be investigated in various ways. From the observed changes in the rate of exudation, conclusions may be drawn concerning the changes in the salt secretion. On the other hand the salt secretion has been investigated more directly by determining the composition and the salt concentration of the exudation sap. A second point for investigation was constituted by the question to what extent salt secretion and exudation would be influenced in circumstances, where active processes should be stimulated (sugar supply) or inhibited (presence of inhibitors in the medium). Our working hypothesis will be discussed in connection with the facts obtained in the experiments.

The investigation was made at the Botanical Laboratory at Groningen. I wish to express my thanks to Professor Dr W. H. Arisz for his valuable advice and the interest taken in my work. To Mr G. C. Mees, Nottingham, I am greatly indebted for his correction of the English text of this paper.

II. MATERIAL AND METHODS

The experiments were performed with tomato plants and Sanchezia nobilis.

Tomato plants about six weeks old were rinsed with water to remove soil particles from the roots. By means of a cork, which had been pierced and split afterwards, the plants were fixed into a hole (diameter 2–3 cm) that had been bored into a wooden disk. The disks were placed on pots containing approximately one litre of Hoagland solution. This nutrient solution contains 0.0025 M. KNO_3, 0.0025 M. $Ca(NO_3)_2$, 0.001 M.$MgSO_4$ and 0.0005 M.KH_2PO_4 in tap water (HOAGLAND and BROYER, 1936). To one litre of solution 1 ml A–Z solution was added. The pH of the solution was adjusted to about 6.2 by adding diluted sulfuric acid. When necessary, that is about every three weeks, iron was given as iron tartrate, 1 ml of a saturated solution to one litre Hoagland solution. In that case a nutrient solution without phosphate was used.

The nutrient solution was replaced every week. If necessary the pots were replenished with tap water in the time between.

In the summer of 1951 the water cultures were placed in a green-house. After 6–8 weeks the plants had developed enough roots to be used in experiments. During the last two weeks the solutions were aerated.

In 1952 the use was obtained of a small green-house, that could be illuminated artificially (VAN DER VEEN, 1950). Thus it was possible to cultivate the plants under more constant conditions, at least in regard to the lighting. Now, too, plants could be cultivated in winter.

For practical reasons the green-house was illuminated from 9 in the evening till 11 in the morning. The temperature rose to 24° C during the light period and fell to 10°–18° C in the dark, depending on the outside temperature.

Owing to lack of room the plants could not be cultivated all the time in this green-house. Usually they were placed in it, when they had been put on a nutrient solution; in winter, however, younger plants still growing in soil were also placed in the green-house. The plants developed well under these conditions, better usually than the plants which were grown in a normal green-house. They could be used after having been on a nutrient solution for 5–6 weeks. The plants showed a stronger exudation and exuded for a longer time, so that often the same plant could be used during two or even three successive days.

Cutlings of Sanchezia nobilis were put on pots filled with tap water after having been fixed in the hole of a wooden disk in the same way as tomato plants. It might take several weeks before roots began to develop. After that the plants were put on a Hoagland solution. In an ordinary green-house the root system had developed sufficiently after 2–4 months depending on the season. In the artificially lighted green-house this took a good two months.

1–3 ml sap was exuded by these plants in an hour. The same plant continued to exude sap for several days, sometimes a week. The plants were cut off some cm above the place where the first roots grow out of the stem. Usually they were decapitated the evening before the day on which the experiment was to take place. Still fixed in the wooden

disk they were placed on a large funnel, whose outlet had been closed by means of a rubber tube with pinchcock. Into the wooden disk two smaller holes had been bored beside the large one. The funnel could be filled through one of them, while the other served for the aeration.

The solution in the funnel could be replaced within one minute. During the night before the experiment the plants were usually put on a Hoagland solution. Next morning this solution was renewed. Once again a piece of the stem was cut off and then a capillary tube was placed on the stump and fixed by means of a rubber tube. The capillary tube consisted of a short vertical and a longer horizontal part, connected by a three-way glass tap. The horizontal part of the tube could easily be emptied by blowing through the tap.

The movement of the meniscus of the sap in the horizontal part of the tube was read off every 30 seconds, with interruptions of a few minutes, if the tube had to be emptied. The tube was calibrated in mm. The reading was estimated to 0.1 mm. One mm corresponded with approximately 1 mm^3 of liquid.

In this way it was possible to follow in detail the changes of the rate of exudation. It appeared from the investigation of ARISZ, HELDER and VAN NIE that the precise course of the change in the rate of exudation could often be of great importance.

Besides the rate of exudation the osmotic value of the sap was determined. Right over the stump a hole was pricked into the rubber tube by which the capillary had been fixed on the stump. A sample of the outflowing sap could be taken through this hole by means of a narrow glass capillary (about 7 cm long, diameter $\pm$ 0.4 mm). If the rubber was sufficiently elastic, the hole closed again on its own account, otherwise it was filled up with some vaseline. Leakage seldom occurred and was perceptible in a short time.

The samples of the sap were preserved in the capillary tubes whose ends had been closed with vaseline, till the osmotic value could be determined. This was done the same day as the samples had been taken.

As a rule the osmotic value of the sap was not determined before a plant had been on a solution for half an hour or more. It appeared that when the circumstances had been altered, it took 20 minutes or more before the osmotic value of the sap was constant again. When the rate of exudation was less, it took more time, before this moment was reached (VAN ANDEL 1952).

The osmotic value of the sap was determined by means of a thermo-electric osmometer according to BALDES and JOHNSON. The principle of this method is as follows: if two drops of solutions of different osmotic values are placed in a container saturated with water vapour, the temperature of the drops is changed to a different extent. The resulting temperature difference between the drops, which is dependent on the difference between their osmotic values, is measured by means of a thermocouple. The apparatus that is used for this purpose, has been described in more detail elsewhere (BALDES and JOHNSON 1939; VAN ANDEL 1952). A small amount of liquid — $\pm$ 0.01 ml — is

required for one determination, so that determinations can be made every now and then, whereas one would have to use up more than 1 ml sap in other methods of determining the osmotic value. A second advantage of this method is, that the determinations take relatively little time. One complete determination takes about one hour, as the drops have to be in equilibrium with their surroundings, but when several thermocouples are available, 5–6 determinations can be made at the same time.

Every thermocouple must be calibrated with known solutions; in this case solutions of boric acid were used. The osmotic value of the sap was always expressed as the concentration of the solution of boric acid, with which it is isotonic. The osmotic values of the external solutions are placed between brackets in this paper. If for instance a 0.005 M. KNO_3 (0.011 M.) solution is referred to hereafter, a solution is meant that contains 0.005 M. KNO_3 and is isotonic with a 0.011 M. solution of boric acid. The determinations proved to be sufficiently accurate. If the osmotic value of the solutions was less than that of a 0.05 M. boric acid solution the error was approximately 0.001–0.002 M boric acid. When the sap or the solutions were more concentrated a larger error was found.

In addition to the rate of exudation and the osmotic value of the sap the water conductivity of the roots was sometimes determined in the way, indicated by ARISZ, HELDER and VAN NIE. According to these authors the factor k in the formula $b = k\,(O_b - O_m)$ should be a measure of its magnitude. When in certain conditions the rate of exudation is b_1, the osmotic value of the sap O_b and that of the medium O_{m1}, $b_1 = k\,(O_b - O_{m1})$. When the osmotic value of the medium is enhanced to O_{m2}, this causes a decrease of b_1 to b_2. Now $b_2 = k\,(O_b - O_{m2})$. This applies to the moment immediately after the change of the medium, when O_b has not yet changed. We can find k now by subtracting the equations: $b_1 - b_2 = k\,(O_{m2} - O_{m1})$ or $k = (O_{m2} - O_{m1})/(b_1 - b_2)$. We know $b_1 - b_2$, the sudden decrease of the rate of exudation. $O_{m2} - O_{m1}$ can be calculated. So k can be determined if a solution of a known osmotic value is replaced by another solution, whose osmotic value is also known.

When sap was to be collected for determinations of the salt concentration, a capillary tube, that had been bent twice at right angles was placed on the stump of the plant. Under the end of the tube a 25 ml container was placed to receive the sap.

The amount of sap, exuded in a certain space of time, was determined by weighing. For determining the concentration of the sap the specific gravity was supposed to be one. The specific gravity proved to be somewhat greater, so the concentrations that have been calculated in this way, are a little too low.

When the amount of sap had been determined the container was topped up with glass distilled water to a volume of 25 ml. The nitrate and phosphate concentrations of the diluted sap were determined colorimetrically: nitrate by the phenoldisulphonic acid method, phosphate according to the molybdenumblue method. Sometimes the

chloride was determined by microtitration according to Volhardt.

The pH of the sap and of the solutions was determined by means of a Beckman pH meter.

In some cases the conductivity of the sap was determined by means of a Philoscoop. The concentrations, determined in this way, showed a good agreement with the concentrations calculated from determinations of the osmotic value of the solutions by means of the thermo-electric osmometer.

In most experiments a Hoagland solution was used, that had been made with distilled water. The solution did not contain iron or A–Z solution. The pH amounted to 5.2. Solutions, that did not contain phosphate, had a somewhat higher pH ($\pm$ 6). In the figures and tables a Hoagland solution is referred to as H, a solution containing no salts but mannitol only as M.

During the experiments the solutions were continually aerated. The experiments were performed in a cellar. The temperature was not quite constant, but showed a rise throughout the day. The changes, however, were very slight. The determinations of the osmotic value were done in the same room, but then a water bath was used, whose temperature could be kept constant.

III. DESCRIPTION AND DISCUSSION OF THE EXPERIMENTS

§ 1. Influence of changes in the osmotic value and the salt concentration of the medium on the exudation

Experiment 1 (fig. 1). A plant was put alternately on a Hoagland solution and on an isotonic solution of mannitol. When the Hoagland solution was changed for one of mannitol, the rate of exudation, which had previously been constant, immediately decreased. The rate of this decrease declined till after about 20 minutes the rate of exudation was again constant. At this time the value was approximately two thirds the original value. The mannitol solution was next replaced by the Hoagland solution: now the rate of exudation began immediately to increase till it was at last just as great as before in such conditions.

It appears from table 1 that the osmotic value of the sap, O_b, was a little lower when the outer solution did not contain any salt, but only mannitol. The product of osmotic value and rate of exudation, which is a measure of the amount of salt given off to the vessels every 30 seconds (S, expressed always as $mm^3 \times M/30$ sec.) showed a decrease. The results of several similar experiments have been summarized in table 1.

Osmotic value and salt secretion into the vessels were determined when the plants had been on a certain solution for about half an hour. As only 0.01 ml of sap was required for a determination by means of the thermo-electric osmometer, the osmotic value of the sap could have been determined sooner, in fact a few minutes after the medium

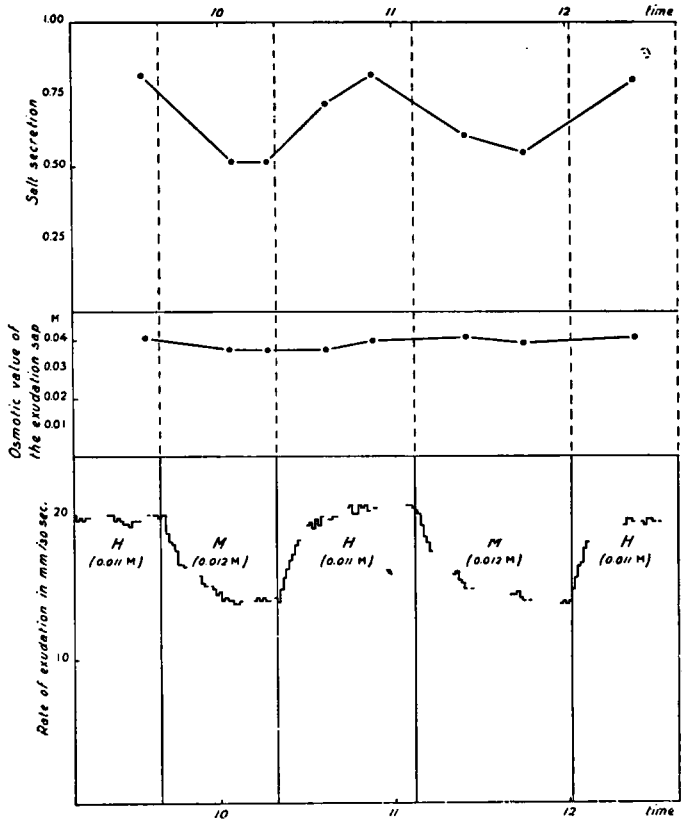

Fig. 1. Changes in the exudation caused by replacing a salt solution by an isotonic solution lacking salts, and vice versa. The rate of exudation is represented in the lower part of the figure; the osmotic value of the sap in the middle part and the salt secretion in the upper part.

TABLE 1

Changes of the salt secretion into the vessels as a result from a change of the salt concentration of the medium. The outer solutions were a Hoagland solution (H) and an isotonic solution of mannitol (M).

Experiment	Medium	H	M	H
1	O_b	0.041	0.037	0.040
	S	0.82	0.57	0.82
	O_b	0.040	0.039	0.041
	S	0.82	0.55	0.82
1*a*.	O_b	0.048	0.041	0.048
	S	0.96	0.54	0.96
1*b*.	O_b	0.042	0.038	0.041
	S	0.81	0.54	0.96
1*c*	O_b	0.044	0.038	0.047
	S	0.66	0.33	0.69
1*d*.	O_b	0.044	0.035	0.042
	S	1.19	0.60	1.08

O_b is expressed as M. boric acid; S as $mm^3 \times$ M boric acid/30 sec.

had been changed. It appeared, however, that it took some time before the exuding sap showed the changes, which had taken place in the sap in the vessels of the roots as a result of changing the medium. About 300–400 mm^3 of sap must flow out before sap of the new concentration reaches the cut surface (VAN ANDEL, 1952).

It appears that if the plants were on a Hoagland solution the amount of salt given off to the vessels was approximately one and a half or two times as large as that which was given off when the outer solution did not contain any salt. This means that the salt secretion diminished considerably if no salt could be taken up, but did not cease altogether. Even when a plant had stayed on a mannitol solution for more than an hour exudation and salt secretion were still very much as they were after the state of equilibrium had been attained.

The rate of exudation was reduced by smaller decreases in the salt concentration of the medium as well as by removing all the salts.

In experiment 2 (fig. 2) the exudation was investigated on solutions containing salts in the same proportion as the Hoagland solution but in different concentrations (0; 0.1; 0.2; 0.5 and 1 H). The solutions had been made isotonic by adding mannitol. Every decrease in the salt concentration of the medium caused a decrease in the rate of exudation. The behaviour was always the same: the rate of exudation decreased rapidly, afterwards more slowly, and became constant after about 15 minutes. The rate of exudation at the state of equilibrium became smaller as the outer solution contained less salt.

The reduction of the rate of exudation was usually accompanied by a decrease of the osmotic value of the sap and therefore by a decrease in the salt secretion too.

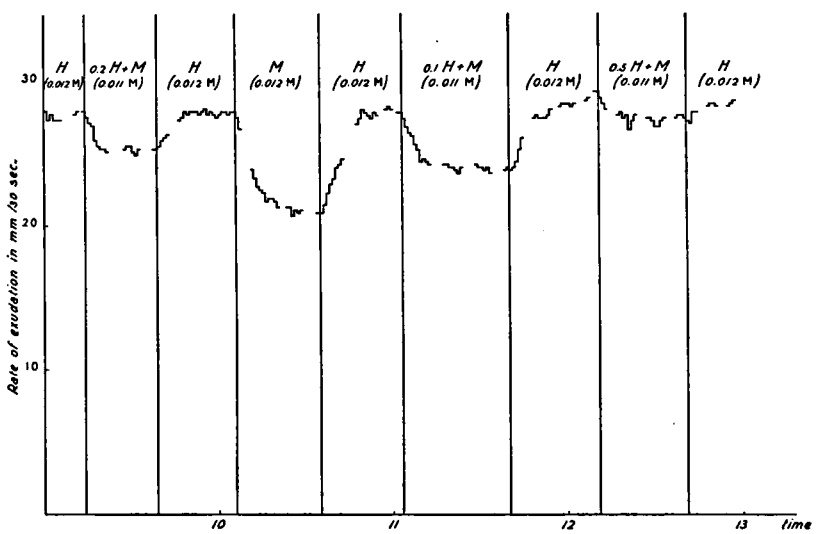

Fig. 2. Relation between the salt concentration of the medium and the rate of exudation.

Changes of the salt concentration of the medium apparently always cause a change of the rate of exudation.

It has been mentioned in the introduction, that changes of the osmotic value of the medium can also cause a change in the rate of exudation. In this case however the changes take another course: enhancing the osmotic value of the medium causes a sudden decrease in the rate of exudation followed by a more gradual increase to a constant level, which is lower than the previous equilibrium value. Conversely the rate of exudation can be suddenly increased by a lowering of the osmotic value of the outer solution, after which a gradual decrease follows (ARISZ, HELDER and VAN NIE). What will happen now, if salt concentration and osmotic value of the medium are changed at the same time?

Experiment 3 (fig. 3). In the left part of the figure we can see the effect of an increase in the osmotic value of the medium, which is just as has been described above. The salt concentration was not

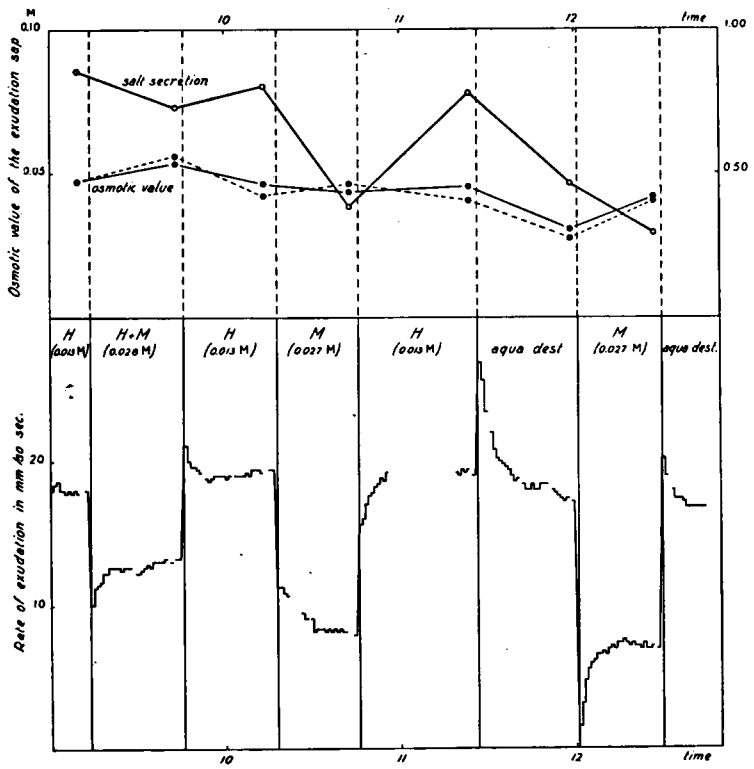

Fig. 3. Influence of changing the osmotic value of the medium on the rate of exudation and salt secretion, compared with the effect of a simultaneous change in the osmotic value and the salt concentration of the outer solution. The rate of exudation is represented in the lower part of the figure; the osmotic value of the sap (●------● experimental data; ●———● data calculated from the formula) and the salt secretion (○———○) in the upper part.

altered in this case. When next the osmotic value of the outer solution was again enhanced but now the salt concentration lowered at the same time by replacing a Hoagland solution by a hypertonic solution of mannitol, the rate of exudation showed a sudden decrease as before. A further more gradual decrease followed the sudden fall. At last a constant level was attained.

Lowering the osmotic value of the medium and addition of salts caused a sudden and then a more gradual increase in the rate of exudation, till the same value had been attained as before on a Hoagland solution.

After this the plant was put on distilled water, which meant a decrease of the osmotic value and of the salt concentration of the medium (sixth period of experiment 3). The sudden increase of the rate of exudation was followed now by a gradual decrease. When next the water was replaced by the mannitol solution the result was the same as when mannitol was added to a Hoagland solution (second period): the rate of exudation was suddenly diminished, but afterwards it increased gradually.

In the first and the last part of the experiment only the osmotic value of the medium was altered; the changes of the rate of exudation took an analogous course in both cases. When, however, the change of the osmotic value was accompanied by a change of the salt concentration as in the third and fourth period, the rate of exudation changed in another way. The equilibrium which was finally attained on a certain solution — a mannitol solution for instance — was the same whether it had been attained by changes of the osmotic value alone, or of the osmotic value and the salt concentration together (fig. 3 fourth and seventh period).

The same phenomenon as has been described just now, is demonstrated in the first part of experiment 4 (fig. 4). In the second part (sixth period) a more concentrated solution of mannitol (0.059 M.) has been used. It appeared that the rate of exudation now changed in a way analogous to that when a solution of mannitol and salts was used (second and eigth period): the rate of exudation decreased first and increased gradually afterwards. The mannitol solution was next replaced by a Hoagland solution. This now caused a sudden increase and gradual decrease.

Another course of the rate of exudation is shown in experiment 5 (fig. 5). In this case a Hoagland solution was replaced alternately by mannitol solutions of different concentrations. The lowest concentration (0.018 M.) caused a sudden decrease followed by a more gradual decrease of the rate of exudation, a phenomenon already known from fig. 3 and 4. The most concentrated solution (0.055 M.) caused the exudation to decrease at first, but afterwards there was a gradual increase just as in fig. 4 (sixth period). When, however, a solution was added whose osmotic value was 0.025 M., the rate of exudation fell suddenly but then stayed at the same level; only after 10 minutes did it decrease somewhat further. On the Hoagland solution (third period) the sudden increase in the rate of exudation

was followed by a further very slight increase. In these experiments the osmotic value of the sap was determined in addition to the rate of exudation. This was done just before the external solution was changed. The osmotic values of the sap in experiments 3 and 4 are summarized in table 2, together with the results of some similar experiments.

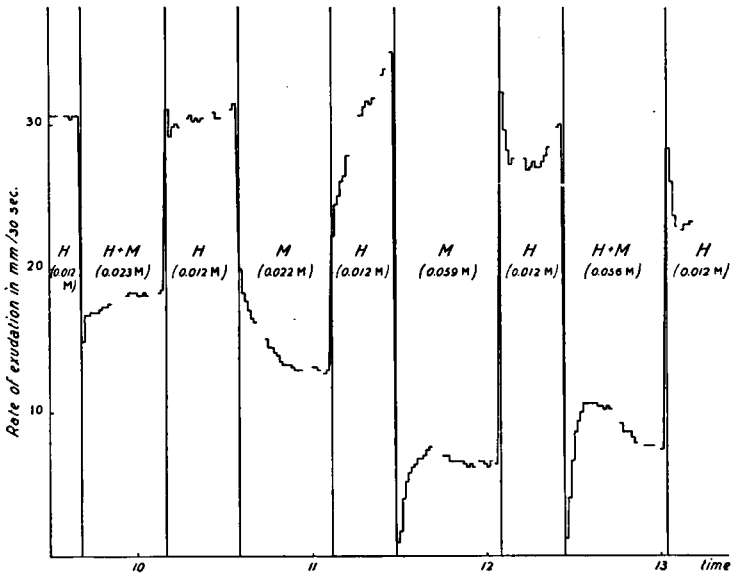

Fig. 4. Comparison between the effects of various changes in the osmotic value of the medium on the rate of exudation in the presence and absence of salts.

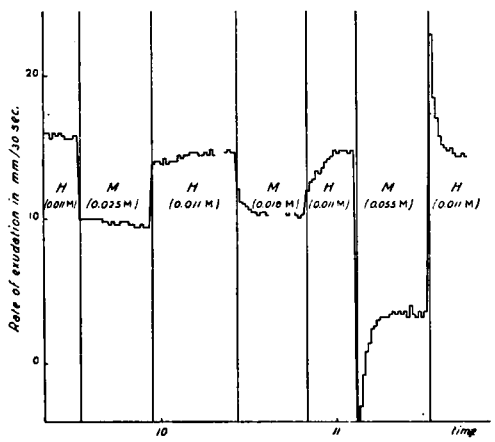

Fig. 5. Relation between the course of the exudation and the change in the osmotic value of the medium. Changes of the osmotic value of the medium are accompanied by changes in the salt concentration, so that water and salt transport are affected simultaneously.

The osmotic value of the sap increased, when the osmotic value of the medium was enhanced, the salt concentration not being changed. Thus the osmotic value of the sap showed a rise from 0.043 M. to 0.053 M. when the osmotic value of the medium was increased from 0.012 M. to 0.023 M. (experiment 4). This agrees with the results of ARISZ, HELDER and VAN NIE. When in the same experiment the osmotic value of the medium was enhanced to 0.022 M. and the salt was removed from the outer solution, the osmotic value of the sap increased, but to a smaller extent, that is from 0.042 M. to 0.049 M. From table 2 it appears that in similar cases the osmotic value of the sap can also stay unaltered (experiment 4*a*, 4*c*) or decrease somewhat.

When a concentrated solution of mannitol was used for the outer solution the osmotic value of the sap always increased, but not to the same extent as when a solution of salt and mannitol of the same osmotic value replaced the Hoagland solution (experiment 4, sixth and eighth period).

In all cases less salt was given off to the vessels, when the outer solution did not contain salt. If we want to compare the values of the salt secretion in different circumstances we must reckon with the fact, that an enhancement of the osmotic value of the medium alone can reduce the salt secretion. Only when the outer solutions differ merely in salt concentration is it possible to get a correct idea about the influence of the salt concentration of the medium on the salt secretion. The influence of the osmotic value of the medium on the salt secretion will be discussed in section 5.

In the experiment described above tomato plants were used. The same results were obtained with Sanchezia nobilis, as can be seen from table 2 (experiment 4*c*). Here also less salt was given off to the vessels when a Hoagland solution was replaced by a hypertonic solution of mannitol. The sudden fall of the rate of exudation was followed by a further more gradual decrease, in the same way that has been demonstrated in fig. 3 and 4 (fourth period).

It may be observed that these phenomena are not due to a specific action of mannitol, since sucrose has just the same effect (experiment 14).

Thus the rate of exudation proves to be influenced by changes in the salt concentration of the medium. The way in which the rate of exudation is changed depends on the extent of the simultaneous change in the osmotic value of the medium.

How are we to explain the phenomena set forth above?

According to the theory of ARISZ, HELDER and VAN NIE there are two possible causes for a change of the rate of exudation. In SABININ's formula $b = k\,(O_b - O_m)$ either k can change or $O_b - O_m$, so that either the conductivity of the roots for water or the difference between the osmotic value of the sap in the vessels and that of the medium must have altered.

Let us first consider the former possibility. When the outer solution is changed k can be determined by dividing the sudden change in the

TABLE 2
Changes in the osmotic value of the sap and the salt secretion caused by simultaneous changes of the salt concentration and the osmotic value of the medium

Exper.	Medium	H	H+M(0.023 M)	H	M(0.022M)	H	M(0.059M)	H	H+M(0.056 M)		
4	O_b	0.043	0.053	0.043	0.049	0.042	0.056	0.042	0.060		
	O_b(theor)	0.046	0.054	0.043	0.037	0.050	0.058	0.058	0.074		
	S	1.32	0.99	1.37	0.64	1.43	0.36	1.26	0.44		
	Medium	H	H+M(0.056 M)	H	M(0.049M)	H	M(0.031M)	H	M(0.073M)	H	M(0.016M)
4a	O_b	0.049	0.067	0.049	0.053	0.047	0.048	0.046	0.061	0.043	0.035
	O_b(theor)	0.059	0.076	0.055	0.067	0.052	0.062	0.057	0.080	0.040	—
	S	1.73	0.92	1.71	0.58	1.66	0.86	1.60	0.51	1.40	0.85
	Medium	H	H+M(0.027 M)	H	M(0.020M)	H	distill. water		M(0.020M)		
4b	O_b	0.026	0.041	0.027	0.033	0.035	0.022		0.030		
	O_b(theor)	0.038	0.041	0.035	0.030	0.043	0.027		0.035		
	S	0.52	0.35	0.46	0.36	0.77	0.51		0.36		
	Medium	H	H+M(0.029 M)	H	M(0.031M)	H					
4c	O_b	0.034	0.043	0.036	0.036	0.036					
	O_b(theor)	0.055	0.072	0.059	0.051	0.062					
	S	0.97	0.92	1.03	0.36	1.00					
	Medium	H	H+M(0.037 M)	H	M(0.037M)	H					
4d	O_b	0.050	0.064	0.048	0.059	0.049					
	O_b(theor)	0.070	0.083	0.071	0.067	0.075					
	S	1.34	1.28	1.32	0.78	1.27					

The osmotic value of the Hoagland solution (H) amounted to 0.011–0.013 M.

rate of exudation by the change in the osmotic value of the medium (see Material and methods).

In experiment 1 isotonic solutions were used so that we cannot calculate *k*. Afterwards, however, the rate of exudation of this plant was determined at different times under various conditions. Then the plant was placed on a more concentrated solution for a few minutes so as to obtain the data necessary for the calculation of *k*. The values found in this way follow below.

Medium	Min. after the change of the medium	Rate of exudation	*k*
Hoagland sol. (0.011 M)		20.4 mm/30 sec.	457
Mannitol sol. (0.012 M)	5	17.4 mm/30 sec.	463
Mannitol sol. (0.012 M)	30	14.6 mm/30 sec.	443
Mannitol sol. (0.012 M)	40	13.8 mm/30 sec.	423
Hoagland sol. (0.011 M)	5	17.4 mm/30 sec.	423
Hoagland sol. (0.011 M)	30	19.4 mm/30 sec.	423

Five minutes after the outer solutions had been changed no alteration in the value of *k* was yet perceptible, although the rate of exudation had already considerably changed at that time.

It appears from experiment 3 too, that *k* is not changed to any considerable extent by the lack of salts in the medium. When considering the values of *k* we must reckon with the influence of the osmotic value of the medium on this factor. The latter phenomenon, which had already been observed by ARISZ, HELDER and VAN NIE, is demonstrated in table 3. For this reason values of *k* can be compared only when solutions of the same osmotic value have been used. In experiment 3 the rate of exudation increased suddenly by 7.8 mm/30 sec when a solution of salts and mannitol was replaced by a Hoagland solution (third period) and when a solution of mannitol was changed for a Hoagland solution the sudden increase amounted to 7.4 mm/30 sec. We can conclude from these data that the values of

TABLE 3
Relation between the osmotic value of the medium and *k*

experiment	medium	H(0.013 M)	H + M (0.037 M)	H(0.013 M)	
4*d*	*k*	516	432	516	
	medium	H(0.012 M)	H + M (0.033 M)	H(0.012 M)	H + Glucose (0.032 M)
15*c*	*k*	411	368	433	356

k in the second and fourth period are only slightly different the change in the osmotic value of the medium being nearly the same in both cases, while the rates of exudation in the condition of equilibrium differed by 5 mm/30 sec.

In some experiments *k* certainly changed. In these cases however the rate of exudation was proved to be different under similar con-

ditions at various times. For this reason these changes are rather to be considered as phenomena of periodicity than as changes of the water conductivity caused by the presence or absence of salts in the medium. Periodical changes of k will be discussed in section 7.

In this case it appears that alterations in the factor k can be left out of consideration as the cause of changes in the rate of exudation. The other possibility mentioned was, that the rate of exudation would be altered as the result of a change in the osmotic difference between the solution in the vessels and the medium.

The osmotic value of the sap depends on the relation between the transport of water and the transfer of salts to the vessels. The water transport is directly dependent on the osmotic value of the medium; the transport of salts is influenced by the salt concentration of the outer solution (table 1 and 2).

The osmotic value of the outer solution remained unaltered in experiment 1, while the salt concentration was lowered. In this case less salt is given off to the vessels, while the amount of water transported there does not immediately change. The sap in the vessels is diluted and the osmotic value must decrease. Consequently the difference between the osmotic value of the sap and that of the outer solution is diminished and so the rate of exudation now falls too. In table 1 we can see, that under such conditions the osmotic value of the sap actually decreased.

In the other experiments the osmotic value of the medium was enhanced and in some the salt concentration was at the same time lowered. An increase of O_m in the formula $b_s = k(O_b - O_m)$ means a reduction of b, that is of the transport of water. The osmotic value of the sap should now increase if the salt secretion were to continue at the same rate. This has been found experimentally when the salt concentration of the outer solution was not lowered (experiment 3, second period; experiment 4, second and eighth period). When however less salt is given off, it is the relation between the decrease in the transport of water and the reduction of the salt secretion that controls the change in the osmotic value of the sap. In experiments 3, 4 and 5 the salt concentration of the medium has been lowered to nought every time, so the salt secretion should always decrease to the same extent. A diluted solution of mannitol would only slightly reduce the water transport, $O_b - O_m$ changing but little. As the salt secretion is reduced considerably, the sap in the vessels will be diluted as in experiment 1, in which the osmotic value of the medium was not altered at all. The rate of exudation slows down as a result of this decrease in the osmotic value of the sap. Salt is carried off from the vessels more slowly now. At last a new equilibrium is established between the rate at which salt is given off to the vessels and that at which it is removed by the flow of water. The osmotic value of the sap does not alter any more and so the rate of exudation is constant too.

Addition of a stronger mannitol solution causes a greater decrease of the water transport. In this case salt that is given off to the vessels is carried off very slowly. It is possible now, that less salt is removed

per unit of time than has been given off to the vessels and the sap must become more concentrated. In consequence more water is taken up and the transport of salts from the vessels is speeded up till an equilibrium has been attained. Here we meet a case analogous to that when only the osmotic value of the medium is changed: in both circumstances the transport of water is reduced as compared with the salt secretion.

The different effect caused by weak and more concentrated solutions of mannitol has been shown in fig. 4 and 5. The changes in the rate of exudation that are caused by a mannitol solution of moderate concentration can be explained in the same way: now the transport of water is reduced by the enhancement of the osmotic value of the medium to the same extent as the salt secretion is reduced because of the lack of salts in the outer solution. The rate of exudation must decrease, but the relation between water and salt transport is not altered and the osmotic value of the sap remains the same. The rate of exudation will neither increase nor decrease after the sudden change.

It will be clear that the changes in the rate of exudation resulting from a decrease in the osmotic value and a simultaneous increase in the salt concentration of the medium can be explained in a similar way. In that case more water is given off to the vessels — $O_b - O_m$ increases — as well as more salt. The flow of water becomes greater as the decrease in the osmotic value in the medium is made greater. The sap will become more dilute or more concentrated according to whether the increase in water transport is more or less than the increase in the salt secretion. Thus after the sudden rise the rate of exudation will either decrease or show a further increase.

It will seldom happen that water transport and salt secretion change to the same extent — which would mean a sudden change only — as this only occurs at one certain osmotic value of the medium. What value this is depends on the salt secretion and the value of k, and these are different for each plant.

A simultaneous decrease in the osmotic value and the salt concentration of the medium must cause an increase in the water transport, but also a reduction of the salt secretion. In consequence the sap is diluted, and after the initial increase the rate of exudation decreases. As a result the exudation changes in the same way as when only the osmotic value of the medium has been lowered. This is demonstrated in fig. 3 (sixth period) at the replacement of a Hoagland solution by distilled water.

Thus we can explain the changes in the rate of exudation caused by changes in the osmotic value and the salt concentration of the medium as the result of changes in water transport and salt secretion. In addition to the osmotic effect of the outer solution, a salt effect can be observed.

If the salt concentration of the medium is lowered while the osmotic value is not much enhanced, the osmotic value of the sap should decrease, as the solution in the vessels becomes more dilute. It has

been pointed out already that in such cases the osmotic value of the sap usually rose, though the increase was smaller when the outer solution contained mannitol alone than when it contained both mannitol and salt (table 2). An actual decrease of the osmotic value has been found only when the salt concentration of the medium was lowered without an enhancement of its osmotic value (table 1).

Conversely the osmotic value of the sap showed a decrease instead of an increase when a weak solution of mannitol was changed for a Hoagland solution.

More concentrated solutions of mannitol caused an increase of the osmotic value of the sap, just as was to be expected.

In our speculation we supposed k to remain unaltered. Now it has been pointed out already that k diminishes if the osmotic value of the medium increases. This could perhaps be the cause of the unexpected increase in the osmotic value of the sap, the water transport being impeded by a decrease of k. When b and k are known we can calculate what osmotic value the sap in the vessels of the roots should have. These values are stated in table 2 (O_b theor.). The increase in the osmotic value of the sap might be explained in this way in a few cases; experiment 3 for instance, where the differences between the values found and those that have been calculated do not exceed the error of the determination.

This deviation from the theory is demonstrated more clearly in experiment 4*d*. On a Hoagland solution the osmotic value of the sap was 0.048 M and on a solution of mannitol 0.059 M (fourth period) so that there was a considerable increase. Osmotic values of 0.071 M and 0.067 M would be expected according to the calculation; that is a decrease.

ARISZ, HELDER and VAN NIE also found a difference between the osmotic value of the exudation sap and the value that had been calculated from the formula. The former was too small. The same thing can be noticed in the example given above (experiment 4*d*). ARISZ, HELDER and VAN NIE mentioned several explanations for this phenomenon, which will be discussed more extensively later. We must state however, that the osmotic value of the exudation sap can also be higher than is to be expected. Thus in experiment 4 (table 2) an osmotic value of 0.049 M was found (fourth period), a value of 0.037 M being calculated. The same thing occurs in experiment 3, but then as has been pointed out already the difference is very small.

Of course experimental errors could be involved. An error can be made in the determination of the osmotic value of the sap as well as in the calculation of k, which is based on the determination of the osmotic value of the outer solutions. However it is improbable that this would explain the difference in experiment 4, the error being only 0.001–0.002 M.

We have pointed to the fact, that it takes some time before changes in the osmotic value of the sap in the roots are shown in the outflowing sap. In experiment 4*a* the fact that the osmotic value of the sap was 0.061 M (eighth period) while the osmotic value of the medium

was 0.073 M, might be explained in this way. But this "retardation" can only account for too low a value after an increase of the osmotic value of the medium, or too high a value when the osmotic value of the medium has been decreased. So we must conclude that in the case of a small increase of the osmotic value of the medium and a decrease in its salt concentration the osmotic value of the outflowing sap can differ from the value calculated from the formula in two ways: the osmotic value changes in a direction opposite to our expectation, and its absolute value can become too high.

The changes in the rate of exudation observed under conditions as set forth above can be explained if we consider the action of the root as that of an osmometer. This working hypothesis, however, cannot account for all phenomena.

We have demonstrated that the decrease of the rate of exudation in the case of a decrease of the salt concentration of the medium, should be considered as the result of the fact that less salt is given off to the vessels. The rate of exudation shows a decrease within one or two minutes after the change of the medium; the same holds true for the increase when the salt concentration of the outer solution is enhanced. It follows from this that the salt secretion changes as soon as the salt concentration of the medium is altered. In experiment 3 the rate of exudation decreased from 19.4 to 11.2 mm/30 sec after an enhancement of the osmotic value of the medium from 0.013 M to 0.027 M. A more gradual decrease followed. This shows that the salt secretion must have diminished to a larger extent than the transport of water. As the latter was reduced by almost 50 %, it must be concluded that the salt secretion decreased to at least one half. The fact that in spite of the large sudden reduction of the water flow a further gradual decrease followed, indicates that considerably less salt was given off to the vessels immediately after the change of the outer solutions. Considering the magnitude of the salt secretion at the equilibrium, we must conclude that almost the whole reduction in the salt secretion took place within one minute after the salt had been removed from the outer solution. Likewise much more salt is apparently given off to the vessels as soon as salt can be taken up from the medium.

Thus the secretion of salt to the vessels is partially held up when no salt can be taken up from the medium. However some salt is still given off and the exudation continues, although at a smaller rate. The necessary salt must come from the tissue. This creates the impression that the process we called "salt secretion" consists of two components. Salt is continually carried from the tissue to the vessels, even when no salt is taken up from the medium. In addition salt taken up from the outer solution can be transported rapidly, and so probably directly, to the vessels. This part of the salt secretion, depending on the salt uptake, will henceforth be called "uptake secretion"; the other part is called "tissue secretion". We may observe that if the expression salt secretion is used, the whole process is meant that achieves the transfer of salts, from the medium or from the tissue, to

the vessels. The expression may be not altogether correct, but is used for brevity's sake.

The tissue secretion causes some transport of water. When the salt secretion is increased in conditions favourable for salt uptake, it is accompanied by an increase in the exudation: the extra exudation could be called "uptake exudation" to distinguish it from the "tissue exudation". It should be understood, however, that these expressions refer to the cause of the transport of water; in both cases part of the water at least comes from the medium.

§ 2. Relation between salt condition of the plant and salt secretion into the vessels

It has been demonstrated in the preceding section that salt can be given off to the vessels even when salt uptake is not possible. This points to the fact that salt is transported from the tissue to the vessels.

Low salt plants are known to have a weak exudation (Broyer, 1951). Barley plants did not show guttation in such conditions. This indicates how important for exudation the salt condition of the roots is.

When a plant had been on distilled water for a day, both salt secretion and exudation were considerably reduced. It seems reasonable to attribute this to the depletion of salts in the root cells. In that case an increase of the salt secretion should be expected when the roots have the opportunity to improve their salt condition by accumulating salts from the medium. This is shown in experiment 6 (fig. 6). A plant was put on a weak salt solution after having been on a solution of mannitol during the previous night (a solution of mannitol was used instead of distilled water to avoid changes in the osmotic value of the medium). The rate of exudation and the salt secretion both increased. Substitution of a solution of mannitol for the salt solution caused a reduction of the exudation and salt secretion, but in the condition of equilibrium both were greater than before. A considerable increase was caused by a stronger salt solution (2 H). When the salts were again removed from the medium, neither

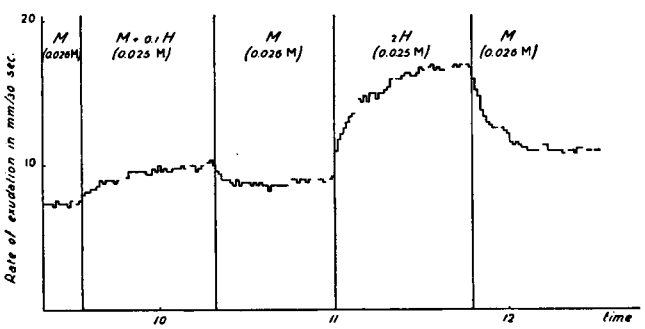

Fig. 6. Increase in the rate of exudation caused by a temporary addition of salt to the outer solution.

did salt secretion nor rate of exudation decrease to the same value as before, although of course some decrease could be observed. The salt secretion increased in this experiment from 0.31 to 0.56, that is by approximately 80 %. From this it can be concluded that the salt condition of the roots must have improved during the periods when salts could be taken up from the medium.

The increase of the salt secretion when salts can be taken up from the medium, can apparently be the result of a direct transport of salts to the vessels as well as of an increased tissue secretion. The pretreatment of the plant appears to be of importance for the tissue secretion. In this experiment however, the uptake of salts had been inhibited for a long time (about 16 hours). In most experiments plants remained on a solution without salts for half an hour only, so that the salt condition of the roots will have been altered less.

§ 3. Influence of the composition of the medium on the exudation

a. Relation between the composition of the medium and the rate of exudation

The rate of exudation proved to be stimulated by the presence of salts in the medium. It may be asked which salts are concerned here. Only meagre data on the influence of various ions on the exudation can be found in literature. In several cases solutions were used containing a mixture of salts; sometimes the different osmotic effect of different salt solutions has been left out of the reckoning. Comparing the exudation of wheat plants on solutions of KNO_3, KH_2PO_4 and $KHCO_3$ with that of plants on distilled water, Lundegårdh (1945) found a decrease of the rate of exudation with however an increase in the concentration of the sap in the first two cases. He concluded from this that nitrate would impede the exudation. The presence of the other salts did not seem to have much influence on the exudation. Raleigh (1946) investigated the influence of salts on guttation. Nitrogen, phosphorus, magnesium, calcium and potassium deficient tomato plants showed no guttation, but guttation could be evoked by adding respectively nitrate, phosphate or potassium to the medium. Addition of calcium or magnesium had no effect. In these experiments however, plants were used which had been grown in solutions lacking one or other element till deficiency symptoms were evident.

To gain an impression of the importance of various anions the exudation was studied when a complete Hoagland solution was replaced by a solution lacking nitrate, phosphate or sulfate. In the first case rate of exudation and salt secretion decreased in the same way as has been shown in fig. 1. In the other cases no change was observed.

These results indicate that nitrates in particular can stimulate the salt secretion. This conclusion can be confirmed by experiments in which the influence of different solutions of single salts was investigated. The concentration of the salts was the same every time. The

solutions had been made isotonic by adding mannitol if necessary. Changing a 0.005 M solution of KNO_3 for a solution of mannitol caused a decrease of the rate of exudation. The rate of exudation was likewise decreased when a solution of $KHCO_3$ or KH_2PO_4 was given instead of the KNO_3 solution. At equilibrium the same level was attained whether the outer solution contained mannitol, phosphate or bicarbonate (fig. 7). The presence of phosphate and bicarbonate seems to have no influence on the exudation, at least in brief

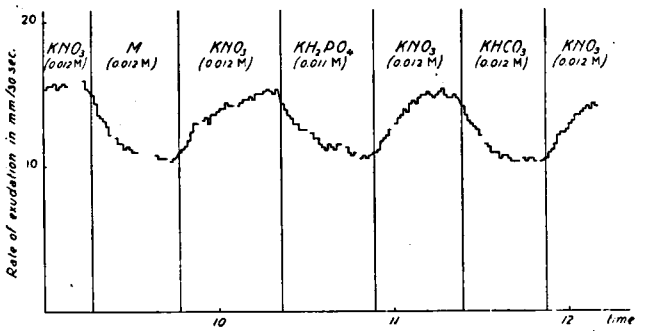

Fig. 7. Changes in the rate of exudation resulting from changes in the composition of the medium.

experiments. The osmotic value of the sap was 0.046 M when the medium consisted of a nitrate solution but 0.040 M and 0.042 M when mannitol and phosphate were present in the outer solution. This means, that in these cases the salt secretion was decreased.

Conversely an immediate increase in the rate of exudation could be observed when a plant was transferred to a solution of potassium nitrate after having been on an isotonic solution of mannitol. If instead of potassium nitrate potassium phosphate was used nothing happened (experiment 8, fig. 8). Potassium chloride could cause an

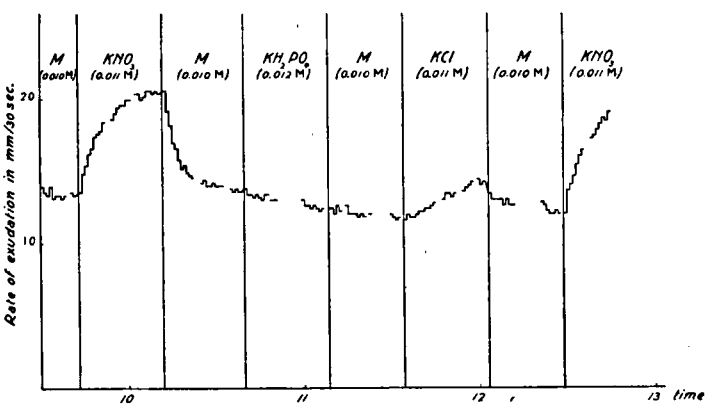

Fig. 8. Increase in the rate of exudation caused by adding KNO_3 or KCl to the medium.

increase in the exudation, but not to such an extent as the nitrate. Even when the roots had been in a solution of potassium chloride for some hours or for a whole night, the exudation did not attain the same rate as when nitrate was present in the medium. A salt effect seems to be caused only by the presence of nitrate or chloride in the outer solution.

We must now investigate to what extent cations are of importance. From the data on water uptake we should expect that potassium and calcium especially would have some influence. It has been stated that in general K promotes the uptake of water, while Ca hampers this process. Most of these results, however, have been obtained from experiments of several days, during which plants were grown under different conditions of salt supply. In these experiments secondary phenomena such as differences of development can be of importance (KISSER, 1927; GASZNER and GOEZE, 1934). However TAGAWA (1934) also demonstrated the influence of K and Ca in a shorter time: decapitated plants of Phaseolus, as well as intact ones, took up more water when the outer solution contained much potassium and little calcium than they did in the reverse case.

SPEIDEL (1939) found a stronger exudation with plants grown in solutions containing little potassium and much calcium than with plants cultivated in conditions of relative Ca deficiency. He supposed that the root cells would give off less water as the result of the swelling of the protoplasm under the influence of potassium. It is known from experiments with intact plants that potassium not only promotes the uptake of water, but diminishes the transpiration as well. LUNDEGÅRDH (1943, 1945) also found a retardation of the rate of exudation if potassium was present whereas calcium had a favourable effect. The differences, however, seem to be small.

We should thus expect that potassium and calcium would perhaps affect not so much the salt secretion as the permeability of the roots for water, which should appear as changes in the factor k.

In table 4 values of the rate of exudation and salt secretion are given, which were obtained when the plants had been on solutions of potassium, calcium, ammonium, or sodium nitrate for half an hour. These values are much the same. No sudden change in the rate of exudation resulting from a change of the medium has been found, so long as the nitrate concentration remains unaltered. Sometimes after a longer period a gradual decrease in the rate of exudation could be observed. This decrease, however, was not the result of any particular change of the medium since it continued during the whole experiment. It might be attributed to the use of an unbalanced salt solution. The same thing could be seen in experiments on the influence of various anions. In most experiments a complete Hoagland solution has been used for this reason, although the presence of nitrate appeared sufficient to cause the salt effect.

The chlorides of various elements were also proved to have the same effect on the exudation (table 4).

It seems that the salt secretion is not much influenced by the nature

of the cations present in the medium. As neither the rate of exudation nor the osmotic value of the sap were changed when a solution of a potassium salt was replaced by a solution of a salt of calcium, sodium or ammonium, we can deduce that k would not change much either. Indeed k was proved to remain unaltered under these conditions.

TABLE 4

Rate of exudation and salt secretion in equilibrium on solutions containing different cations. The solutions contained 0.005 M salt and were isotonic.

Experiment	Medium	KNO_3	$NaNO_3$	KNO_3	NH_4NO_3	KNO_3	$Ca(NO_3)_2$
9. . . .	*b*	10.6	10.6	9.8	8.6	8.4	8.6
	S	0.46	0.42	0.39	0.33	0.33	0.33
	Medium	KNO_3	$Ca(NO_3)_2$	KNO_3	$Ca(NO_3)_2$		
9*a*. . .	*b*	16.6	16.4	15.0	14.6		
	S	0.75	0.73	0.60	0.57		
	Medium	KNO_3	$NaNO_3$	KNO_3	KCl	KNO_3	NH_4NO_3
9*b*. . .	*b*	11.4	12.2	12.4	10.6	12.0	11.8
	S	0.48	0.52	0.48	0.40	0.46	0.44
	Medium	KCl	$CaCl_2$	KCl	NH_4Cl	NaCl	KCl
9*c*. . .	*b*	8.4	7.8	8.0	7.0	7.2	7.4

Even when a plant had been for twenty four hours on a solution containing only potassium nitrate or calcium nitrate, nothing happened when this solution was replaced by the opposite one of calcium or potassium nitrate respectively. In similar experiments, however, there is the difficulty that the exudation can change spontaneously over such a long period.

b. Concentration and composition of the exudation sap in relation to the composition of the medium

Different opinions exist concerning the relation between the concentration of the sap and that of the medium. According to SABININ (1925) the concentration of ions in the sap is independent of their concentration in the outer solution. The amount of every ion present in the sap should be characteristic for that ion. Thus he found potassium and phosphate in the sap in higher concentrations than in the outer solution, while calcium and ammonium were found in lower concentrations. LAINE (1934) on the contrary stated that the concentrations of cations (potassium, calcium and magnesium) in the sap was proportional to their concentration in the medium: $s = kc^{1/n}$, s being the concentration of the ion in the sap, c that in the medium and k and n constants. LUNDEGÅRDH (1943, 1945) found that an increase in the concentration of potassium nitrate in the outer solution caused a large increase in the concentration of potassium as well as of nitrate in the sap. When the outer solution contained calcium nitrate the salt concentration of the sap was only slightly increased, when the concentration of the medium was enhanced. The rate of exudation decreased because the salt concentration of the outer solution was greater so that in this case the amount of salt given off to the sap

did not alter. If the solution contained potassium nitrate actually more salt was given off as the salt concentration increased. LUNDEGÅRDH did not therefore find any definite relation between the concentration of a salt in the medium and that in the sap, although some influence could be demonstrated.

On account of the influence of the osmotic value of the medium on that of the sap, which has been demonstrated in section1, it is necessary to use isotonic solutions when investigating the relation between the salt concentration of the medium and that of the sap. LAINE's solutions were not isotonic but their concentrations were rather low, so that their osmotic effect may have been small. In LUNDEGÅRDH's experiments solutions of different concentrations had also different osmotic values; LUNDEGÅRDH, however, considers the osmotic effect of the medium on the sap concentration to be of no importance. It is difficult to evaluate these results.

Even if we eliminate the osmotic effect of different solutions some difficulties arise. As we argued before, the concentration of the sap depends on the relation between the transport of water and the transfer of salts to the vessels. The first is, among other things, influenced by k, a factor that differs with each plant. The transfer of salts is influenced by changes in the salt concentration of the medium, as has been pointed out in section 1, but it also depends on the salt condition of the roots. Moreover the concentration of the sap was not the only factor found to change when the salt secretion was altered, for the amount of sap given off to the vessels per unit of time also varied. This means a change in the rate of water transport, which must again influence the salt concentration in its turn. Finally the salt secretion is influenced not only by the salt concentration of the outer solution but also by its osmotic value. It will be clear that so many factors can influence the concentration of the sap, that the possibility of representing the relation between its salt concentration and that of the medium by a simple formula is as LUNDEGÅRDH (1945) has already stated, hardly to be expected.

We have seen that one part of the salt secretion depends on the possibility of taking up salts from the medium. As the latter process will depend on the concentration of the salts in the medium, it might be expected that some relation would be found between the concentration of the medium and this part of the salt secretion. For reasons mentioned above, this concerns not changes in the concentration of the sap but changes in the amount of salt given off to the vessels per unit of time.

In table 5 the results are to be seen of some experiments done to determine this point. The part of the salt secretion depending on the uptake of salts from the medium is calculated by subtracting the amount of salt given off when no salt is available in the medium, from the salt secretion after the addition of salt to the outer solution. Only in one case could any regularity be observed in the increase of the uptake secretion with the increase of the salt concentration of the medium. Irregularities can be attributed to several factors. The

most important is the periodicity of salt secretion. It appears that the tissue secretion changes during the day. For a discussion of this phenomenon readers are referred to section 7. If we therefore calculate the uptake secretion by subtracting the tissue secretion found at one time from the whole amount of salt given off at another time, incorrect

TABLE 5
Relation between the salt concentration of the outer solution and the salt secretion (on isotonic solutions)

Medium	Salt secretion	in mm³ × M/30 sec.	
	experiment 2	2*a*	2*b*
Hoagland 100 %	1.26	1.04	1.01
„ 0 %	0.65	0.90	0.61
„ 10 %	0.90	—	0.66
„ 20 %	1.04	1.01	0.83
„ 30 %	—	—	0.58
„ 50 %	1.16	1.08	0.77
„ 200 %	—	1.22	—
„ 100 %	1.35	—	0.72

values will be obtained. To obtain reliable osmotic values for the sap it is necessary that the plants shall be on a certain solution for at least half an hour, so that the influence of so few concentrations can be investigated that it is impossible to draw any conclusions as to the relation between the salt concentration of the medium and the amount of salt given off to the vessels.

The importance for the exudation of the presence of nitrate ions in the medium leads to the supposition that they should compose an important part of the ions in the sap. LUNDEGÅRDH (1943, 1945) found indeed mainly potassium nitrate in the sap of wheat plants. Cl, PO_4, SO_4, NH_4 and Ca were present in very small amounts. Organic substances have seldom been found in the sap of herbaceous plants. Large amounts of sugar such as appear in the sap of various trees do not seem to occur (VAN OVERBEEK, 1942; SKOOG, BROYER and GROSSENBACHER, 1938). LITVINOV (according to LAINE) only, mentions the presence of relatively large amounts of organic acids and organic nitrogen compounds in the exudation sap of pumpkins.

Much nitrate was found in the exudation sap of tomato plants. The presence of phosphate, carbonate, or bicarbonate, and a very little sulfate could also be demonstrated. Chloride was found only when the plant was on a solution of chloride, while the other ions were also found in the sap of plants that had been on distilled water for some time.

Potassium as well as calcium and ammonium seemed always to be present, potassium probably predominating.

We did not succeed in demonstrating the presence of sugars or amino acids.

Approximately the same results were found with plants of Sanchezia nobilis, but the sap of this plant seemed to contain more chloride; that is to say that chloride could also be demonstrated in

the sap of plants that had been grown on a Hoagland solution made with tap water, which contains always some chloride.

The results of several determinations of the concentrations of nitrate and phosphate in the sap are summarized in table 6. These determinations were made at different times of the day (the salt secretion changes during the day as will be shown later) and in different circumstances so that the roots were on isotonic solutions with or without salts. Sap was collected during periods of approximately two hours. The amount of sap was too small to determine the concentration of other ions beside nitrate and phosphate. It is not, however, to be expected that other ions were of much importance. From a comparison of the osmotic value of the sap and its nitrate concentration it appears that other anions could only have been present in very small amounts, while we found earlier that the various cations are not of great importance for the exudation.

The nitrate concentration of the sap always exceeded that of the Hoagland solution. By the evening less nitrate was given off to the vessels while the exudation of sap likewise decreased. Next morning both nitrate and sap were given off again in greater amounts.

The sap contained much more nitrate than phosphate. The secretion of phosphate into the vessels changed proportionately more during the day than the nitrate secretion, but the changes usually coincided. Like LUNDEGÅRDH we found that under these conditions the sap consists chiefly of a solution of nitrate, probably potassium nitrate.

When a plant was transferred from a Hoagland solution to an isotonic solution of mannitol less sap was exuded and less nitrate was given off to the vessels. Nitrate however remained the main constituent of the sap even when no salt could be taken up from the medium. When decapitated plants had been on a mannitol solution or distilled water for 36 hours the amount of nitrate given off to the vessels per hour might still be approximately one third of the quantity secreted while the plant was on a Hoagland solution.

If a solution without salts was replaced by a Hoagland solution more nitrate was given off to the vessels and the exudation of sap also increased.

The quantity of phosphate in the sap appeared to be influenced less by changes of the medium. Sometimes even more phosphate was given off while the plants were on a mannitol solution or distilled water for 2–4 hours, than during periods in which phosphate could be taken up (table 6). This could lead to a considerable increase in the phosphate concentration of the sap, the rate of exudation being diminished as a result of the smaller nitrate secretion. Conversely the secretion of phosphate into the vessels did not always increase when phosphate could be taken up from the outer solution as was the case with nitrate. Also when the uptake of salt was inhibited for a longer time the secretion of phosphate was not so much affected as that of nitrate. The sap of a plant which had been on distilled water for twenty four hours after decapitation contained about three quarters

TABLE 6

Quantity of sap and amounts of nitrate and phosphate given off during two days on a Hoagland solution or an isotonic solution lacking salt

time	medium	g. sap/hour	osmotic value sap	concentration of nitrate	amount of nitrate m.e./hour	concentration of phosphate	amount of phosphate m.e./hour
			Experiment 10. Tomato plant				
8.15–10.15	Hoagland	2.539	0.049 M	0.024 M	0.061	0.0010 M	0.0026
10.15–12.15	Hoagland	2.722	0.051 M	0.023 M	0.063	0.0016 M	0.0044
12.15–14.15	Hoagland	2.810	0.050 M	0.022 M	0.062	0.0016 M	0.0046
14.15–16.15	Hoagland	2.666	0.047 M	0.022 M	0.059	0.0017 M	0.0046
16.15–18.15	Hoagland	2.682	0.045 M	0.019 M	0.051	0.0018 M	0.0049
18.15– 8.15	Hoagland	1.945	0.034 M	0.015^5 M	0.030	0.0012 M	0.0023
8.15–10.15	Hoagland	2.449	0.034 M	0.017 M	0.042	0.0011 M	0.0026
10.15–12.15	Mannitol	1.331	0.034 M	0.018 M	0.024	0.0018 M	0.0024
12.15–14.15	Mannitol	1.143	0.035 M	0.014^5 M	0.017	0.0015 M	0.0017
14.15–16.15	Hoagland	2.045	0.033 M	0.017^5 M	0.036	0.0012^5 M	0.0026
			Experiment 10a. Sanchezia nobilis				
8.15–10.15	Hoagland	3.888	0.033 M	0.018 M	0.068	0.0004 M	0.0016
10.15–12.15	Hoagland	3.992	0.034 M	0.018 M	0.072	0.0004 M	0.0016
12.15–14.15	Hoagland	4.010	0.033 M	0.018 M	0.071	0.0004 M	0.0016
14.15–16.15	Hoagland	3.990	0.032 M	0.015 M	0.060	0.0004 M	0.0017
16.15–18.15	Hoagland	4.036	0.032 M	0.014 M	0.057	0.0003 M	0.0011
18.15– 8.15	Hoagland	4.200	0.025 M	0.014 M	0.057	0.0002 M	0.0008
8.15–10.15	Hoagland	4.058	0.030 M	0.015 M	0.061	0.0003 M	0.0011
10.15–12.15	Hoagland	3.934	0.031 M	0.016 M	0.063	0.0002 M	0.0012
12.15–14.15	Mannitol	2.005	0.027 M	0.012 M	0.023	0.0007 M	0.0014
14.15–16.15	Mannitol	1.843	0.021 M	0.011 M	0.020	0.0009 M	0.0017
16.15–18.15	Hoagland	3.540	0.027 M	0.013 M	0.056	0.0004 M	0.0015

the amount of phosphate that had been given off on a Hoagland solution, while the quantity of nitrate was decreased to one third.

These data are for the sap of tomato plants (table 6, experiment 10) as well as that of Sanchezia nobilis (table 6, experiment 10*a*).

Fig. 9 also demonstrates the great influence of the uptake of salts on the secretion of nitrate into the vessels. In these experiments solutions of 0.005 M KNO_3, KH_2PO_4, KCl or a solution of mannitol were used. A small quantity of $CaSO_4$ was added to the first three solutions to counteract an eventual injurious effect from the potassium ions. The solutions were isotonic.

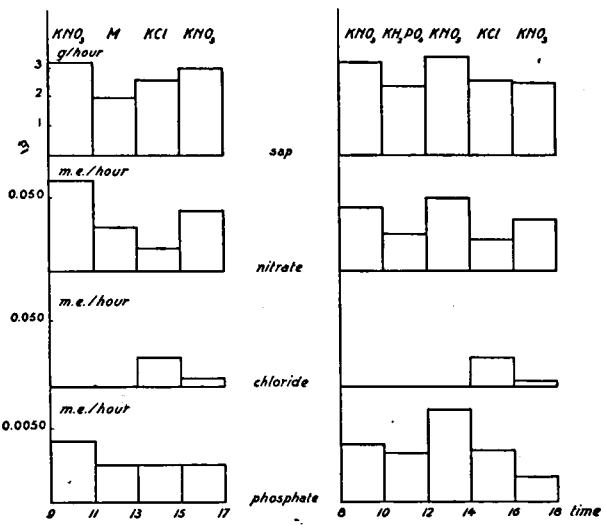

Fig. 9. Comparison of the amounts of sap, nitrate, chloride and phosphate given off on isotonic solutions containing KNO_3, KCl, KH_2PO_4 or mannitol.

Exudation of sap and secretion of nitrate decreased considerably every time the plants were transferred to a solution lacking nitrates. Addition of nitrate to the medium caused an increase of both.

When the plants could take up chloride from the medium, chloride was found in the sap in considerable quantity. This was accompanied by an increase in the exudation of sap as has already been demonstrated in fig. 8. This is seen most clearly when a solution lacking salts is replaced by a solution of potassium chloride.

The large decrease in secretion of chloride when potassium chloride was again removed from the medium is remarkable. It appears that chloride, like nitrate, can be transported directly to the vessels after having been taken up from the medium. It seems however, that only a small quantity of chloride goes from the tissue to the vessels. This suggests that chloride is present in the tissue in small quantities only, which would mean that the greater part of the chloride taken up from the outer solution is transported directly to the vessels. The results of VAN NIE (unpublished) are in favour of this supposition.

The amount of chloride present in the sap of plants which had been on distilled water for some days and had then been put on a solution of potassium chloride and decapitated, did not show any further increase after the first few hours. When a considerable amount of chloride had been transported to and accumulated in the tissue an increase of the tissue secretion and so of the whole salt secretion might have been expected as we have seen in experiment 6 (fig. 6). A similar case arises when a low salt plant is put on a solution of potassium nitrate: the amount of nitrate given off to the vessels and the exudation continue to increase for more than twenty four hours. Of course before this could be stated with certainty it would be necessary to compare uptake and secretion of chloride at the same time.

In experiment 11 (fig. 9) the amount of phosphate given off to the sap did not change when a solution of potassium nitrate was replaced by a solution of potassium phosphate during two hours. In the next period however when the phosphate was absent again from the medium, more phosphate was given off to the vessels. In another experiment, not recorded here, the same has been found; the secretion of phosphate this time increased after the substitution of a phosphate solution for a nitrate solution, but a further increase followed in the next period, when phosphate was absent from the medium. This indicates that while phosphate is taken up from the medium, it can take a rather long time before it reaches the vessels. The transport of phosphate to the vessels seems to be much slower than that of nitrate or chloride. This conclusion is consistent with the fact that phosphate could not stimulate the exudation when a mannitol solution was replaced by a solution of phosphate (fig. 8).

It seems that phosphate is transported to the vessels in some other way to nitrate and chloride. The small influence of the phosphate concentration of the medium on the amount of phosphate given off to the vessels may indicate that only the so-called tissue secretion transfers phosphate to the vessels. The secretion will then remain unaltered so long as sufficient phosphate is available in the tissue.

We have already concluded, from a comparison of the osmotic value of the sap with its nitrate concentration, that nitrate remains the main constituent of the sap when no salt uptake takes place. This was checked by determining the conductivity of the sap (nitrate being supposed to be present as potassium nitrate), the same conclusion being reached from the results.

To explain the gradual increase in the rate of exudation after the addition of mannitol to the medium EATON (1943) supposed mannitol to permeate into the roots. It has been pointed out already in the Introduction that this supposition appears to be superfluous. From the determinations of the salt concentration of the sap we must now conclude that there really is no evidence for the permeation of mannitol into the vessels. The increase in the osmotic value of the sap following a rise in the osmotic value of the medium is caused by an increase in its salt concentration.

In a few cases, however, the osmotic value of the sap did not con-

form to the nitrate concentration. When the osmotic value of the sap, which could be calculated from its nitrate content, was higher than the value that had actually been found, the differences were always small, so that they might be accounted for by experimental errors. It has been pointed out in the chapter Material and methods that the concentration of the sap calculated from the amount of nitrate and phosphate found may be somewhat too high. If we calculate the osmotic value of the sap from the amount of nitrate present, supposing all nitrate to exist as fully dissociated potassium nitrate, we shall obtain too high a value if in fact some of the nitrate is present as the calcium salt.

Several times the sap contained less nitrate than was to be expected from its osmotic value, especially when the plant had been for a longer time on a medium lacking salts (a solution of mannitol as well as distilled water). The difference could amount to one fourth of the total osmotic value (0.01 M boric acid), but it was usually not so large.

This would indicate that other substances can also be present. The presence of phosphate could not account for the difference. Electrolytes seem to be concerned as the conductivity of the sap was also greater than was to be expected from the nitrate concentration.

We have not succeeded in identifying this substance or substances. As carbon compounds were proved to be present, it seemed most plausible that the unknown substance would be carbonate, bicarbonate, or an organic acid. Sugar and amino acids are not present in demonstrable quantities as has been mentioned before. According to LUNDEGÅRDH (1945) no organic acids can be found in the sap of wheat plants, but bicarbonate can be demonstrated in considerable quantities. Under certain circumstances he found a shift of the relation between cations (K^+) and anions (NO_3^-) resulting from a shortage of the latter. In his opinion bicarbonate would be compensating in this case. He confirmed this supposition by determinations of the bicarbonate concentration of the sap.

We have tried to gain an impression of the nature of the unknown substance by making titration curves of the sap.

The pH of the sap itself was between pH 5.5 and 6.0. It could rise to 6.8 after one or two days.

The titration curves were made in the following way: 15 ml of sap was titrated with diluted HCl, the pH having been adjusted first to 10.5–11 by adding NaOH. The results are to be seen in fig. 10, in which is represented the relation between the pH of the sap and the amount of dilute acid necessary to shift it by half a unit. The buffer capacity of the sap proved to be largest between pH 6 and 7 and pH 9 and 10. In all curves a maximum was found in those ranges. The buffer capacity was, however, very small between pH 3.5 and 5.5. Were organic acids, such as for instance malic acid, present a good buffering at low pH values would be expected. This was verified by making a titration curve after the addition of a small quantity of malic acid to the sap. In this case the sap was actually much more

buffered at low pH values. This points to the fact that if such acids are present, their quantity must be very small.

The buffering at pH 6–7 can be explained partially by the presence of phosphate. In fig. 10 next to the titration curve of the sap a titration curve of 15 ml of a phosphate solution of the same concentration is represented. This solution proves to be not so well buffered, which points to the presence of another substance. When a small amount

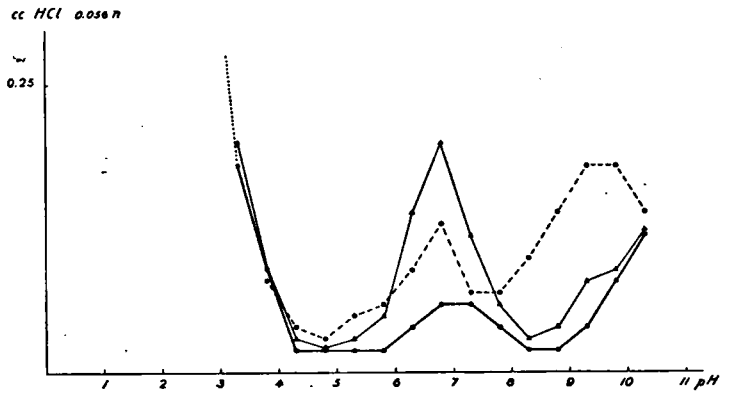

Fig. 10. Relation between the quantity of HCl required to shift the pH by half a unit and the pH. ●------● sap; ○———○ solution of KH_2PO_4 containing as much phosphate as the sap; ▲———▲ solution of phosphate and 0,001 M. $KHCO_3$.

of potassium bicarbonate (0.001 M.) was added to this solution, a stronger buffering was found between pH 6 and 7, stronger in fact than was shown by the sap itself. So it seems that the amount of bicarbonate present in the sap must be very small, and probably not sufficient to account for the difference between nitrate concentration and osmotic value.

c. Influence of the pH of the outer solution on the exudation

The exudation of wheat roots is not according to LUNDEGÅRDH (1949) very sensitive to changes of the pH of the medium; less sap was exuded only at very low (3) or high (9) pH values. There are few data on the influence of pH on water uptake by roots. However it is known from many experiments that the permeability to water of pieces of tissue can be influenced very much by changes in the pH of the outer solution. The results of these experiments are not always in accordance. This may be attributed partly to differences between the methods used and partly to the fact that different objects have been investigated (DRAWERT, 1952). The water permeability was usually found to be maximal at a not too acid pH and to decrease at lower and higher values (VON GUTTENBERG and MEINL, 1952).

The pH of the solutions used in the preceding experiments varied between 5.2 and 6.0, so we thought it important to investigate to

what extent changes in the pH of the medium could possibly be concerned with the observed changes of the rate of exudation. Special attention was paid to changes of k, although we must bear in mind that k, the conductivity of roots for water, need not be identical with the permeability to water of the pieces of tissue mentioned above. In the latter case the rate at which water goes from the medium into the vacuole is determined (transmeability according to ARISZ, 1945) while if intact roots are concerned, we investigate the rate at which water goes from the medium to the vessels, either passing through the cytoplasm or even through the cell walls.

The rate of exudation proved to decrease when a Hoagland solution (pH 5.2) was replaced by a similar solution whose pH had been increased to 7.2 or decreased to 3.4 by the addition of KOH or H_2SO_4. The decrease in the rate of exudation set in several minutes after the change of the pH. The exudation decreased continuously at about the same rate and even after one hour no constant level had been attained. The rate of exudation increased gradually when these solutions were replaced again by a Hoagland solution with a pH of 5.2.

Changes in the pH of the outer solution seemed to have little influence on the exudation so long as the value did not drop below 4.5 or rise above 6.5. More acid or alkaline solutions caused the exudation to decrease. This decrease was accompanied by a decrease of k. This can be seen in table 7. The salt secretion was not influenced much by the changes in the pH of the medium: as the rate of exudation decreased the osmotic value of the sap increased, sometimes to twice its initial value. The data in table 7 (experiment 13) indicate a small decrease of the salt secretion, but it may be questioned if this is a real decrease. At the moment the osmotic value of the sap was determined no equilibrium had been attained, the rate of exudation was still decreasing and so the osmotic value was probably rising. The osmotic value of the outflowing sap might have been lower than that of the sap in the vessels of the roots at the same time. In this way the value of the salt secretion would turn out to be too small.

TABLE 7

Influence of the pH of the medium on rate of exudation (b); osmotic value of the sap (O_b); saltsecretion (S) and conductivity for water of the roots (k). All values were determined when the plants had stayed on a certain solution for one hour.

Medium	pH	b	O_b	S	k
Hoagland solution	5.20	9.4	0.043	0.40	170
„ „	3.25	3.5	0.091	0.32	66
„ „	5.20	9.1	0.047	0.43	166
„ „	7.50	8.5	0.050	0.43	170

The decrease in the rate of exudation caused by changes in the pH of the medium was always completely reversible. The value of k increased again, while the osmotic value of the sap showed a decrease.

When a dilute acid or alkali was used, that is when no salts were present in the medium, the same results were found.

It is probable that the changes of the pH of the medium are of no

importance to the phenomena described in the preceding sections, as the differences of pH were rather small. It appears moreover that changes in the rate of exudation resulting from changes in pH follow another course to changes induced by alterations of the osmotic value or the salt content of the outer solutions.

§ 4. Stimulation of the exudation under the influence of sugar

The exudation of tomato plants has been shown to increase if the plants can utilize more sugar (Went 1944). It has been pointed out already that an increase in the rate of exudation can have two causes; either the factor k must become greater, or a greater amount of osmotically active material must be given off to the vessels. In this case the first possibility would not seem very probable and indeed will prove to be unimportant.

Lundegårdh (1945) on the contrary found a decrease in the rate of exudation on the addition of glucose to the medium. Less salt was given off to the vessels; because in Lundegårdh's opinion more nitrate would be assimilated in the roots when more sugar was available.

Went applied sugar to the plants by submerging two leaves left on the stump in a solution of sucrose. This method meets with some difficulties, as the effect of sucrose administered in this way is perceptible only after twelve hours. For this reason we added sugar to the medium. Using this technique Went could find no effect, but on the other hand White (1938) and Street and Lowe (1950) have found that tomato roots are able to take up sucrose from a nutrient solution. It may be observed that in these experiments as in our own the medium consisted of a solution, whereas Went used plants grown in soil which was sprinkled with a solution of sucrose.

The influence of sucrose was first investigated as it was known from the investigations just mentioned that it is taken up by the roots of tomato plants. Addition of sucrose to the outer solution, however, meant an increase in the osmotic value of the medium. To meet this difficulty rather low concentrations of sucrose were used. Moreover the exudation on a solution to which sucrose had been added was always compared with that on an isotonic solution containing mannitol instead of sucrose.

Experiment 14 (fig. 11). The Hoagland solution was first replaced by one that had been made isotonic with the solution containing sucrose, so that it would be possible to gain some idea of the changes due to a purely osmotic effect. When the plants had been on a Hoagland solution again for some time, sucrose was added, while salts were still present. At first the effect was the same as when a solution of mannitol was used: the rate of exudation fell suddenly and afterwards increased gradually. This effect must be considered purely osmotic. After approximately 20 minutes, at a time when equilibrium should have been attained, the rate of exudation showed a gradual increase, while after a good hour, approximately the same rate was attained as had previously been recorded on a Hoagland solution (whose

osmotic value is lower). The rate of exudation amounted to 27.2 mm/30 sec at this time, while it was only 13.4 mm/30 sec when the plant had stayed on a mannitol solution for an equal period.

When the solution containing sucrose was changed again for a Hoagland solution, the rate of exudation rose suddenly by a large amount — osmotic effect — but afterwards an unusual strong and prolonged decrease followed. After one hour no equilibrium had yet been attained, while as a rule this takes about fifteen minutes.

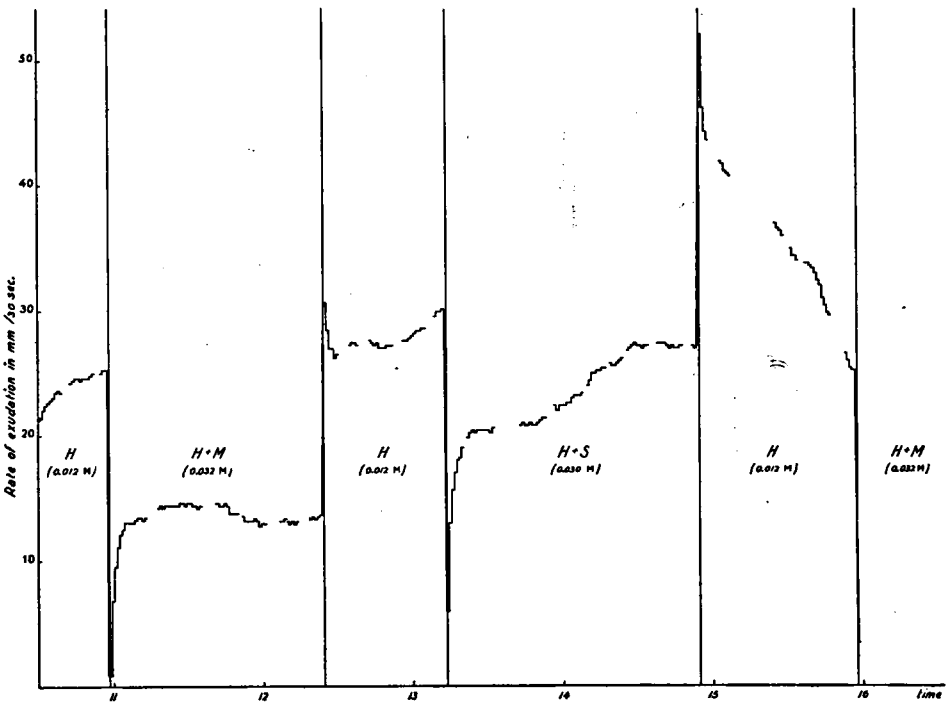

Fig. 11. Effect of adding sucrose (0,02 M.) to a Hoagland solution on the rate of exudation, compared with the effect of mannitol.

When the osmotic value of the medium was lowered the sudden rise in the rate of exudation was approximately the same whether sugar or mannitol had been used. This indicates that the stronger exudation in the presence of sugar cannot be attributed to a change of *k*. The osmotic value of the sap in the condition of equilibrium was 0.035 M when the medium consisted of a solution of salt and mannitol, and 0.038 M after a stay on the solution with sucrose. Although the difference was small, a greater amount of osmotically active substances must have been given off to the vessels in the latter case to cause the stronger exudation. Thus the salt secretion — or more in general the secretion of osmotically active substances — proves to be stimulated by the presence of sucrose in the medium.

We can explain the unusual course of the exudation in the last period of experiment 14 in this way: the salt secretion was promoted at first because of the sucrose that had been taken up into the roots; as the supply of sugar gave out the salt secretion and rate of exudation decreased. This pattern of behaviour proves that the increase on the solution of sucrose is not caused by periodicity.

Such a strong effect as in this experiment could not always be observed. This is shown in table 8 for instance, where the results are given for an experiment in which the osmotic effect of the sucrose solution had been eliminated, as the plant was put alternately on isotonic solutions containing salts and either mannitol or sucrose. The favourable effect of sucrose on the exudation in this experiment is not great but it is unmistakable.

The exudation of plants grown in daylight was usually more

TABLE 8
Influence of sucrose on rate of exudation and salt secretion

	time	*b*	S
H + Mannitol (0.022 M.)	11.00	11.5	0.43
„ „	11.30	10.3	0.44
„ „	12.00	9.2	0.35
H + Sucrose (0.022 M.)	12.30	9.1	0.35
„ „	13.00	10.8	0.40
„ „	13.30	12.5	0.50
„ „	14.00	13.0	0.47

influenced by sugar than that of material cultivated in the artificially lighted green-house. The latter was generally in a better condition; the sugar content of the roots may have been higher as a result of the better lighting.

The effect of sucrose could always be observed only after 20–30 minutes. It apparently takes some time before sufficient sugar has been taken up in the roots to cause an effect.

Experiment 15 (fig. 12). As in the preceding experiment the effect on the exudation of a solution containing sucrose was compared with that of a mannitol solution; in this case however, the solutions of sucrose and mannitol lacked salts. We must conclude that it now makes no difference whether the Hoagland solution was replaced by a solution of sucrose or one of mannitol: the change observed is the result of the simultaneous enhancement of the osmotic value and reduction of the salt secretion of the medium. When sucrose was present (fourth period) the rate of exudation was even a little smaller than in the condition of equilibrium on mannitol. The periodicity might account for this: the rate of exudation showed a tendency to decrease throughout the experiment, as is apparent if we compare the rate of exudation in the first and the third period, when the medium consisted of a Hoagland solution.

When the solution of sucrose was next replaced by a Hoagland solution the rate of exudation showed a sudden increase followed by a more gradual one, as would be expected for a decrease in the

osmotic value of the medium accompanied by an increase in its salt concentration. The gradual increase, however, was much greater than when the salt solution was substituted for a mannitol solution, whereas we should expect a smaller increase because of the periodicity. An equilibrium was not attained but a strong decrease in the rate of exudation set in after approximately 20 minutes. It seems plausible to attribute this phenomenon to the action of sugar taken up from the medium in the preceding period. This would mean that sugar acts as soon as salt can be taken up. At first the exudation is stimulated but as in experiment 14 the exudation must decrease again as the supply of sugar runs out.

It appears that the rate of exudation was not influenced by the sugar so long as the outer solution did not contain salts, nor was the secretion of osmotically active substances promoted. The results of

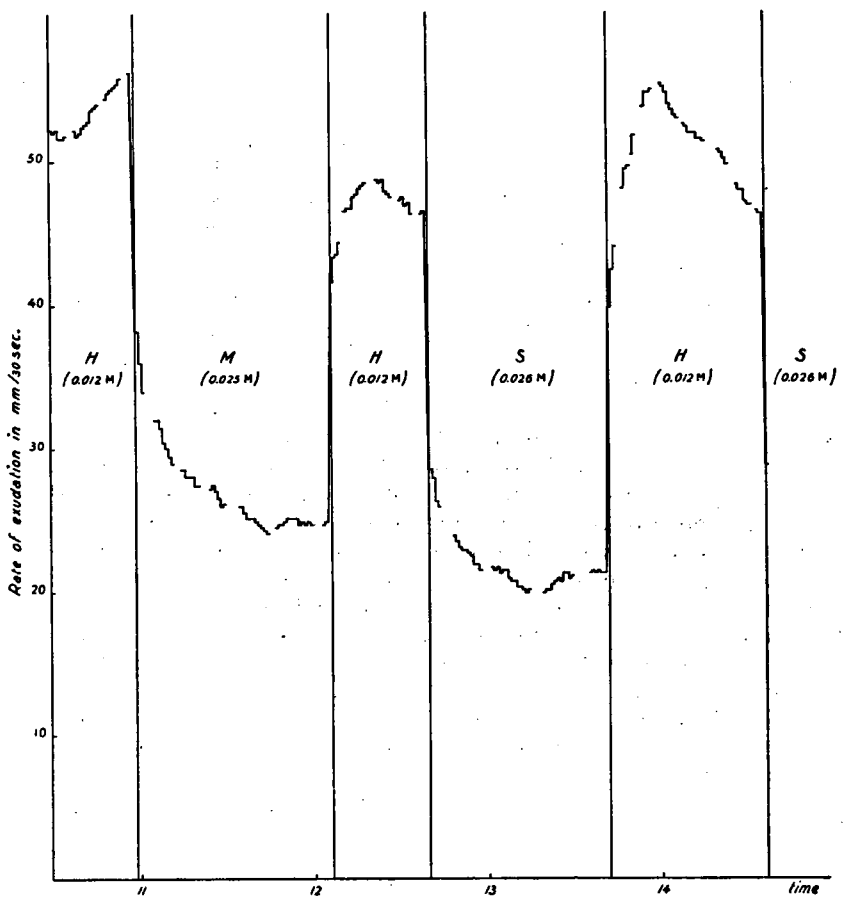

Fig. 12. Changes in the rate of exudation caused by replacing a salt solution by a solution of sucrose or mannitol, lacking salts.

two similar experiments have been recorded in table 9. We must emphasize that a stimulating effect of sucrose has only been found in cases when the outer solution contained both salt and sugar. This points to the fact that sucrose acts on the secretion of salts into the vessels. It is not probable that sucrose permeates into the vessels and is the cause of the stronger exudation, as this would also happen in the absence of salts. This was investigated by examining sap for the presence of sugars by means of the reaction with α-naphtol and sulfuric acid as well as by following the method of Hagedorn-Jensen. No sugar was found, even when the plants had been on a sucrose solution for several hours.

TABLE 9

Influence of sucrose on rate of exudation and salt secretion in solutions lacking salt as compared with the influence of mannitol

Experiment	Medium	H (0.015 M)	M (0.015 M)	H (0.015 M)	Sucrose (0.014 M)	H (0.015 M)
15*a*	*b*	21.6	15.2	23.4	14.4	25.6
	S	0.86	0.48	0.90	0.37	0.73
	Medium	H + M	M	M + Sucrose	H + Sucrose	
15*b*	*b*	20.6	7.0	3.2	22.6	

We did not succeed in demonstrating any stimulation by adding glucose to the medium.

The exudation of Sanchezia plants was not influenced very much by the addition of sucrose. Glucose could also exercise some influence. The sugars seemed to produce that effect only when salts were present in the medium, but because of the smallness of the stimulation it was difficult to state it with certainty.

That sucrose seemed to influence the exudation only when the outer solution contained salts beside the sugar was rather unexpected. First we must consider the possibility that the presence of salts is of importance for the uptake of sucrose. STREET and LOWE (1950) stated that the uptake of sucrose by the roots of tomato plants was favoured by the presence of phosphate. This would explain why we could not find an effect of sucrose when salts were lacking. It does not, however, seem very probable, that this is so because of the unusual course of the exudation when salts were added to the outer solution after the plant had been on a sucrose solution. This indicates as we explained above, that sucrose has been taken up while salts were absent.

It is difficult to explain this action of sucrose on the exudation. It might be that the uptake of salts from the medium is promoted. It is known that the salt uptake is related to the sugar condition of the roots. This has already been pointed out by HOAGLAND and BROYER (1936). STEWARD and PREVOT (1936) found that high salt barley roots could take up as much bromide as low salt, high sugar plants if glucose was added to the medium. It has been shown in several investigations with barley (HUMPHRIES 1951) as well as with maize (VAN ANDEL, ARISZ and HELDER 1950; HELDER 1952) that more phosphate was taken up if glucose was added to the medium.

According to HUMPHRIES the uptake of nitrate would also be stimulated. The last fact might be of importance here, as we should expect that an increased secretion of nitrate especially would be the cause of the increase of the exudation. In most investigations just mentioned however, it was the accumulation of ions in the root cells that was stimulated in particular, and we do not know if this also means an increase in the secretion of salts into the vessels. If we suppose that the increase in the exudation is caused by a stimulation of the salt uptake, then it seems curious that it takes so much time before this effect is noticeable, whereas the presence of salts always influences the exudation immediately. We will return to this point later.

§ 5. CHANGES IN SALT SECRETION AS A RESULT OF ALTERATIONS OF THE OSMOTIC VALUE OF THE MEDIUM

When considering table 2 we can observe that the salt secretion appears to decrease every time the osmotic value of the medium is enhanced, even when the salt concentration is not changed. More salt proves to be given off if the osmotic value of the medium is lowered again.

The reduction was sometimes considerable. Thus the salt secretion was reduced to 75 % in experiment 4, when the osmotic value of a Hoagland solution was enhanced to 0.023 M (second period). When the osmotic value of the medium was still more increased (eighth period) a reduction to 35 % could be observed. The last value however, may be somewhat too small because of the periodical decrease of the salt secretion, since on a Hoagland solution also less salt seemed to be given off. However other experiments also show that the salt secretion is progressively reduced as the osmotic value of the medium is further enhanced. This can also be seen in the following experiment (experiment 16). The salt secretion was determined on various non-isotonic solutions of different salt concentrations. The increase of the salt concentration was now accompanied by an enhancement of the osmotic value of the medium. The salt secretion did not increase (compare experiment 2). However if we compare the amount of salt given off to the vessels while the plant was on a concentrated salt solution (2 H) with that given off under the same osmotic conditions but at a lower salt concentration (0.5 H + M) we see that the higher salt concentration still has some favourable effect.

Experiment 16:

	0.5 H (0.012 M)	2 H (0.030 M)	0.5 H (0.012 M)	0.5 H + M (0.031 M)	0.5 H (0.012 M)
S	0.83	0.76	0.89	0.61	0.87

It seems that there are two opposite effects: on the one side the salt secretion is stimulated by the higher salt concentration; on the other hand it is decreased by the higher osmotic pressure of the medium. This could mean that the uptake secretion is stimulated as the result of the higher salt concentration of the medium while the

tissue secretion is hampered; but it can also be imagined that both processes are hampered, so that the uptake secretion is not stimulated to the same extent as at lower osmotic values.

ARISZ, HELDER and VAN NIE also found an inhibition of the salt secretion at higher osmotic values of the outer solution. They supposed that more energy would perhaps be required to secrete salt into the vessels, as the sap would become more concentrated. If this were true an inhibition of the whole salt secretion should be expected.

Uptake and tissue secretion were determined in outer solutions of different osmotic values. To this end we determined how much salt was given off to the vessels when a plant was on a Hoagland solution, an isotonic solution of mannitol and a Hoagland solution again. This gave the value of the tissue secretion and the uptake secretion could also be calculated. The plant was then transferred to a Hoagland solution whose osmotic value had been enhanced by the addition of mannitol. After half an hour this solution was replaced by an isotonic solution of mannitol only, which was changed in its

TABLE 10

Changes in salt secretion resulting from changes of the osmotic value of the medium

Experiment	Osmotic value medium	Amount of salt given off on a Hoagland solution	Amount of salt given off on an isotonic mannitol solution	Difference (... uptake secretion)
16*a*.	0.014 M	0.70[5]	0.41	0.29[5]
	0.041 M	0.49[5]	0.23	0.25[5]
	0.014 M	0.71	0.42	0.29
16*b*.	0.014 M	1.56[5]	1.00	0.56[5]
	0.052 M	1.26	0.71	0.55

turn for the solution of salt and mannitol, again. At the end of the experiment the plant was put once more on an ordinary Hoagland solution to see whether there had been any changes in the rate of exudation or salt secretion as a result of periodicity. There were no such variations in experiments 16*a* and *b*, so the values found for uptake and tissue secretion may be considered reliable. It was now possible to calculate the values of uptake and tissue secretion when the outer solution had a higher osmotic value. The uptake secretion proved not to change when the osmotic value of the medium was enhanced from 0.014 M to 0.052 M, as it amounted to 0.565 in the first case and to 0.55 in the latter. The tissue secretion however showed a decrease from 1.00 to 0.71 (table 10). It appears that in particular less salt is given off from the tissue when the osmotic value of the outer solution is higher. The transport to the vessels of salts taken up from the medium does not seem to be affected.

Although the osmotic value of the sap in this experiment increased to 0.067 M from 0.042 M on a Hoagland solution and 0.039 M on a solution of mannitol, this cannot be the cause of the decrease in the salt secretion.

It is possible to influence one part only of the salt secretion in this way. This supports our supposition that two different processes are involved.

The same phenomenon, of a decrease in the secretion of salts into the vessels as the result of higher osmotic values of the medium, is to be seen in LUNDEGÅRDH's tables (1945). Less potassium and nitrate was given off to the sap at higher glucose concentrations of the outer solution. LUNDEGÅRDH attributed this to a stimulation of the nitrate assimilation. However the same thing occurred when the concentration of potassium or calcium nitrate in the medium was very high, whereas smaller enhancements of the salt concentration stimulated the secretion of salts. This seems comparable to our experiment 16. We will discuss LUNDEGÅRDH's explanation later.

§ 6. THE INFLUENCE OF INHIBITORS ON THE EXUDATION

In preceding sections the hypothesis has been advanced that two different processes may be involved with the transfer of salts to the vessels. We have tried to gain a better insight into their mechanisms through influencing the salt secretion by means of inhibitors.

a. Potassium cyanide

It is known, that the exudation can be inhibited by the addition of KCN to the medium. According to VAN OVERBEEK (1942) a certain part of the exudation of tomato plants can be inhibited reversibly. ROSENE (1944) also stated that the exudation of Allium roots suffered an inhibition under the influence of KCN at higher concentrations (0.001 M). Lower concentrations could exercise a stimulating influence. ROSENE supposed that two processes would be involved, one of which was sensitive to KCN while the other was not; for the rate of exudation at first decreasing under the influence of the cyanide, after some time stayed at the same level. This hypothesis of ROSENE's does not necessarily imply an active and an inactive (that is osmotic) process, as VAN OVERBEEK thought. LUNDEGÅRDH (1949, 1950) found that the exudation of wheat plants was at first promoted; but that the rate of exudation decreased if the inhibitor was allowed to act for a longer time.

Experiment 17 (fig. 13). It was our intention to investigate the influence of KCN on the tissue exudation as well as on the part of the exudation depending on the presence of salt in the medium. The exudation was first examined under normal conditions: rate of exudation and salt secretion were compared when a plant was on a Hoagland solution and on an isotonic solution of mannitol. When, on a Hoagland solution again, an equilibrium had been attained KCN (0.0005 M) was added. This caused the rate of exudation to decrease immediately. The rate of decrease slowed down and after 25 minutes the rate of exudation was almost steady. At that time a new solution, again a Hoagland solution with 0.0005 M KCN, was added, for the concentration of KCN can diminish rapidly in a non-

alkaline solution that is well aerated. This might be the reason why the rate of decrease was slowing down. When the outer solution had been renewed the rate of exudation did indeed decrease somewhat further.

After the plant had been on a solution of KCN for one hour, an investigation was made to see whether a salt effect could still occur: The Hoagland solution was replaced by a solution of mannitol con-

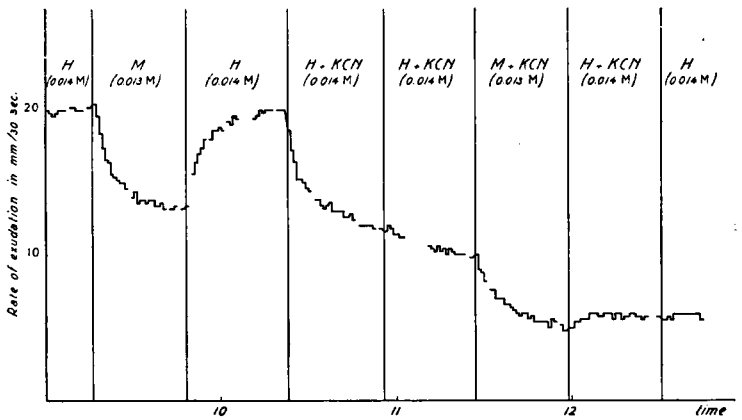

Fig. 13. Changes in the rate of exudation caused by the addition of 5.10^{-4} M.KCN to the outer solution. Comparison of the salt effect under normal conditions and in the presence of KCN.

taining as much KCN and the resulting decrease in the rate of exudation indicated that the uptake secretion was still active; but when, half an hour later, a Hoagland solution (with KCN) was given once again, the rate of exudation showed hardly any increase.

The rate of exudation proves to decrease under the influence of KCN. The course of this decrease bears a remarkable resemblance to that caused by the removal of salt from the medium. One might be inclined to suppose that an inhibition of the uptake secretion would be involved here. However, the situation seems to be less simple than this. In fig. 13 we can see how the rate of exudation is diminished to 12 mm/30 sec when KCN is added to a Hoagland solution. The rate of exudation amounted to 13 mm/30 sec when no salts were present in the medium (second period). This indicates that tissue exudation must have been partially inhibited at any rate, even if the exudation depending on the uptake of salts had been inhibited completely. The latter supposition proved however to be false, since the removal of salts induced a further decrease of the rate of exudation (sixth period). We must however point out that the decrease caused by the replacement of a salt solution by a solution without salts appears greater than it really is. The rate of exudation was still decreasing at the moment when the salts were removed (sixth and seventh period). The big decrease then observed must be attributed to the change of the medium, but the value attained in the condition

of equilibrium was controlled by two factors: the decrease in the rate of exudation resulting from the lack of salts, and the prolonged inhibition under the influence of KCN. This is indicated by the fact that the rate of exudation did not increase very much when, afterwards, salts were added again (eighth period.) The osmotic value of the sap decreased when the salt concentration of the medium was diminished, but otherwise it did not show any changes. It appears that when KCN is added to the medium less salt is given off to the vessels; because the osmotic value of the sap did not change we must conclude that the reduction of the salt secretion may not be the only cause of the decrease in the exudation, since if in the formula $b = k\,(O_b - O_m)$, b decreases while $O_b - O_m$ is not changed, k must decrease too. In this experiment k was not determined. Fuller reference to this point will be made later.

The exudation was only partially inhibited in this experiment. After approximately one hour and a half the exudation did not seem to decrease any further, although we must reckon with the possibility that the concentration of KCN was in the meantime reduced. It appeared to be possible to inhibit the exudation completely by using higher concentrations (0.001 M) but this took several hours although a decrease set in as soon as KCN had been added to the outer solution.

A stimulation of the exudation was never observed with the concentrations used (0.0001–0.005 M). Big differences appear to exist between the sensitivity of individual plants to KCN. The material used in 1951, that had been grown in an ordinary green-house, was on the whole less sensitive than the plants which were used later and which had been cultivated in the artificially lit green-house. In the first case a concentration of 0.005 M KCN might not cause a complete inhibition, the inhibition being furthermore fairly well reversible. When the more sensitive plants were used the exudation recovered partially or not at all when KCN was removed from the medium. It may however be observed, that BROUWER (1953) found that it took sixteen hours before intact plants of Vicia Faba recovered from treatment with KCN. Our experiments were not continued so long.

It has been already mentioned that experiment 17 indicates not only that less salt is given off to the vessels, but also that k is changed under the influence of KCN. This was investigated in more detail in the following way: the changes in the rate of exudation were not followed continuously, but the average rate of exudation during five minutes was determined every half hour. A sample of sap was taken at the same time to determine its osmotic value. Then the plant was put on a more concentrated solution for a few minutes so that k could be calculated from the sudden decrease in the rate of exudation. The data of such an experiment are given in table 11. The rate of exudation decreased, if KCN was added to the medium, but the osmotic value of the sap remained constant, the salt secretion therefore changing to the same extent as the rate of exudation. K also showed a decrease. Conversely the increase in the rate of exudation after the removal of KCN, was accompanied by an increase of the salt secretion as well as of k.

The exudation of Sanchezia nobilis also decreased on the addition of KCN. The course of this change was identical to that described in experiment 17: the decrease was rapid at first, but afterwards slowed down without altogether ceasing during the experiment. Tissue as well as uptake secretion was inhibited although a distinct salt effect could occur still one and a half hour after KCN had been added.

TABLE 11
Effect of KCN and DNP on rate of exudation, salt secretion and conductivity for water

Exp.	Medium	time	b	O_b	S	k
17*a*	Mannitol	10.30	11.8	0.023	0.27	840
	,,	11.00	11.6	0.022	0.26	800
	0.005 M KCN	11.30	7.6	0.019	0.14	540
	,, ,,	12.00	7.0	0.020	0.14	560
	Mannitol	12.30	9.0	0.020	0.18	620
	,,	13.00	8.6	0.022	0.19	660
	0.005 M KCN	13.30	6.4	0.021	0.13	360
18*a*	distilled water	10.15	6.5	0.020	0.13	241
	,, ,,	10.45	7.5	0.024	0.18	289
	10^{-4} M DNP	11.15	6.0	0.022	0.13	241
	,,	11.45	4.5	0.021	0.09	144
	distilled water	12.15	4.7	0.020	0.09	151
	,, ,,	12.45	5.1	0.020	0.10	187
	,, ,,	13.15	6.4	0.020	0.12	244

The osmotic value of the sap did not change, so that here k must have decreased too. Both exudation and salt secretion proved to recover fairly well, when the solution of KCN was removed.

So we have found that KCN can inhibit the exudation. The inhibition proved in some cases to be complete so that there is no reason to suppose that a certain part of the exudation process is not affected, although we found that after some time the rate of exudation did not decrease further when weak concentrations were used. The course of the decrease was very much like that caused by an inhibition of the salt secretion resulting from the removal of salts from the medium. The decrease however, cannot be explained in a similar way, its causes are complicated. It has been pointed out already that the tissue secretion is inhibited besides the uptake secretion. This is clearly demonstrated when KCN is added to a solution without salts: the rate of exudation then decreased in exactly the same way as when in fig. 13, KCN was added to a Hoagland solution (fourth period). The whole process of salt secretion must be concerned here. We cannot say, if this decrease in the salt secretion takes place immediately KCN is added, because the decrease then observed is caused not only by a reduction of the salt secretion and the consequent decrease in the osmotic value of the sap, but also by a decrease of the water conductivity of the roots, the factor k.

Although both uptake and tissue secretion were inhibited, it is shown by fig. 13 that the first is inhibited to a greater extent. The salt effect had become very small two hours after KCN had been

added. While the rate of exudation on a solution without salts had decreased from 13.2 mm/30 sec to 5 mm/30 sec, the tissue secretion itself had decreased in that time from 0.52 to 0.21. This perhaps is not so surprising since it is to be expected that the uptake of salts will be completely inhibited by a similar concentration of KCN. MACHLIS (1944) for instance found, that the uptake of bromide by barley roots was fully inhibited after a treatment of two hours with KCN. 0.0001 M. KCN would according to LUNDEGÅRDH entirely inhibit the anion respiration of wheat roots which controls the uptake of anions. Again WEEKS and ROBERTSON (1950) found with carrot tissue an inhibition of salt uptake by KCN.

b. 2.4-Dinitrophenol

The qualitative action of 2.4-dinitrophenol did not prove to differ much from that of KCN. Like KCN it caused a decrease in the rate of exudation that set in immediately, both when the outer solution contained salts as when it did not. The osmotic value of the sap was not changed. It follows from this, that the salt secretion changed to the same extent as the rate of exudation. The tissue secretion must certainly be inhibited as DNP affected the exudation when no salts were present in the medium. The influence of DNP on the part of the exudation that depends on the uptake of salts was investigated in the same way as in experiment 17: with the same plant the salt effect was investigated under normal conditions and in the presence of DNP. Fig. 14 shows that both uptake and tissue secretion were diminished. Rate of exudation and salt secretion decreased to approximately one half during the experiment. After the inhibitor had been removed from the medium, the rate of exudation increased gradually: but after two hours it had not yet completely recovered.

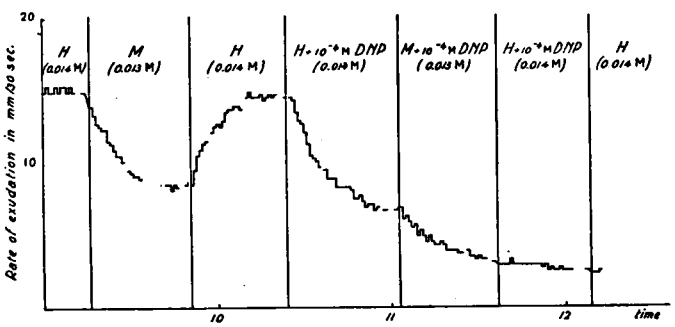

Fig. 14. Influence of dinitrophenol on the salt effect.

It is difficult to say to what extent the exudation is influenced in these experiments by the periodicity. The fact that the rate of exudation was very regular at the beginning — before addition of the inhibitor — indicates that periodicity is not very important here. Periodical changes were rather small with plants grown in the artificially illuminated green-house as will be demonstrated in section 7.

In these experiments such plants were chiefly used, so it is probable that the decrease in the rate of exudation, salt secretion and k, is for the greater part at least caused by the inhibitor, although the possibility of small periodical changes especially at the end of the experiment, cannot be ruled out altogether.

The sensitivity of plants to DNP proved to be rather variable. The same concentration that was used in experiment 18 (fig. 14) caused in other cases an inhibition of 15–20 % only, which was completely reversible.

It has been pointed out already that the osmotic value of the sap did not change under the influence of dinitrophenol. A decrease of k should therefore be expected. Table 11 (exp. 18*a*) shows that this was actually the case.

It is known that DNP uncouples respiration and phosphorylation processes. The uptake of salts by carrot tissue is inhibited by DNP (ROBERTSON, WILKINS and WEEKS, 1951), but as we explained while discussing the action of KCN, a decrease of the salt uptake cannot be the only cause of the decrease in the exudation. ARISZ (1953) found that the uptake of phosphate and asparagine by tentacles of Drosera was inhibited. ROBERTSON, WILKINS and WEEKS mentioned the possibility of an inhibition of the transport of salts by DNP. This would explain the simultaneous inhibition of uptake and tissue secretion. It should be clearly understood however, that the experiments of ROBERTSON, WILKINS and WEEKS concern the transport of salts to the vacuole and our experiments the transport to the vessels.

ARISZ (1953) did not find any influence of DNP on the transport of chloride through the cytoplasm of Vallisneria, but the secretion of salt into the vacuole proved to be inhibited.

c. Sodium arsenate

Experiment 19 (fig. 15). Addition of 10^{-5} M sodium arsenate to the medium (distilled water) caused a decrease of the rate of exudation. This decrease set in immediately and passed off in the same way that has been described in the experiments with KCN and DNP. The osmotic value of the sap did not change, so in this case too less salt must have been given off — from the tissue —, and the water conductivity of the roots must have decreased. The exudation did not recover when the solution of Na-arsenate was replaced after half an hour by distilled water. From distilled water the plant was transferred to a Hoagland solution. After 40 minutes this solution was changed for distilled water again. Now the rate of exudation proved to have increased to its initial value. Addition of Na-arsenate — a solution ten times more concentrated this time — caused the exudation to decrease once again.

The inhibition caused by Na-arsenate appears to be reversible, but it is remarkable that the exudation recovered only after the plant had stayed on a salt solution for some time.

WARBURG and CHRISTIAN (1939) found in experiments on isolated enzyme systems that arsenate can replace inorganic phosphate in

oxidative reactions, for which the uptake of inorganic phosphate is necessary. BONNER (1950) stated that the growth of Avena coleoptiles induced by indole acetic acid was inhibited by Na-arsenate, but that this inhibition could be overcome by adding phosphate to the medium. In the light of these data it seemed probable that the reversal of the arsenate inhibition described in experiment 19 would be the result of the presence of phosphate in the Hoagland solution. It has indeed been proved that exudation and salt secretion did not recover on a

Fig. 15. Reversible decrease of the rate of exudation caused by adding sodium arsenate.

solution containing all the salts from a Hoagland solution with the exception of phosphate. On the other hand no decrease of the exudation could be observed if arsenate was added to a complete Hoagland solution; if a Hoagland solution without phosphate was used the exudation decreased (the absence of phosphate by itself is unimportant c.f. section 3*a*). For this reason a Hoagland solution without phosphate was used in the experiments designed to investigate the influence of arsenate on exudation.

It has been shown in experiment 19 that the tissue secretion decreased on the addition of Na-arsenate to the outer solution. The influence of Na-arsenate on the part of the exudation governed by the uptake of salts was investigated in the way described before. In fig. 16 it is demonstrated that the rate of exudation decreased from 19.4 mm/30 sec to 13.6 mm/30 sec when a Hoagland solution was changed for a solution of mannitol. The addition of 10^{-4} M Na-arsenate caused a very small decrease, that could be completely reversed, but 10^{-3} M arsenate made the rate of exudation decrease from 18.4 to 14.4 mm/30 sec and the rate of exudation showed a further decrease to 10.6 mm/30 sec if the Hoagland solution was now

replaced by a mannitol solution (also containing arsenate). The osmotic value of the sap changed only when the salt concentration of the medium was lowered. Thus the osmotic value decreased from 0.042 M to 0.038 M in the second period and from 0.042 M to 0.036 M, when the Hoagland solution was replaced by the mannitol solution in the presence of arsenate. This means that both tissue and

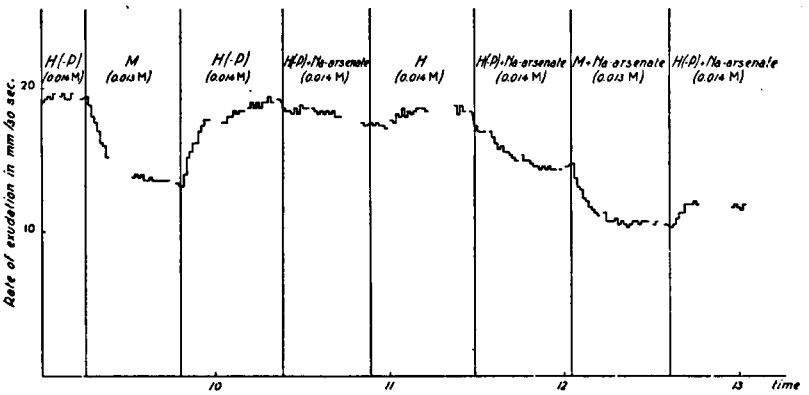

Fig. 16. Influence of sodium arsenate on the rate of exudation in the presence and absence of salts. In the fourth period 10^{-4} M sodium arsenate was added, in the sixth and following periods 10^{-3} M.

uptake secretion are diminished. This conclusion could have been drawn already from the fact that the removal of salts caused a smaller decrease of the exudation if arsenate was present, but in that case we must reckon with the possibility that k has become smaller. The same decrease of the salt secretion could then cause a smaller decrease in the rate of exudation.

In addition to the changes of rate of exudation and salt secretion a decrease of k has been demonstrated.

d. Sodium fluoride

LUNDEGÅRDH (1949, 1950) found that the exudation of young wheat plants was inhibited by NaF in concentrations of 0.01–0.001 M. The osmotic pressure of these solutions was considered unimportant. LUNDEGÅRDH first concluded that the sensitivity of the exudation to NaF would depend on an exudation of cations to the vessels. This process would be controlled by glycolysis. Later experiments (1950) lead to another conclusion, since it appeared that the rate of exudation, that is the amount of water given off to the vessels, did indeed decrease under the influence of NaF, but the secretion of nitrate did not. The nitrate concentration of the sap even proved to show a great increase. LUNDEGÅRDH now supposed that the exudation of salts was not linked to anaerobic glycolysis but that it was independent of metabolism. The decrease in the exudation of sap would be the result of an inhibition of the so-called "extra-water" secretion,

the exudation of water released in metabolic processes. This extra water could in his opinion amount to 2/3–3/4 of the total sap.

LUNDEGÅRDH's experiments were repeated with tomato plants.

Most experiments were carried out with solutions containing 0.005 and 0.01 M NaF, at a pH of 5.2. The trouble about using such high concentrations in our experiments is that the solutions impede the exudation on account of their osmotic value. This is shown in table 12. The rate of exudation decreased when 0.01 M NaF was added to a salt solution, while the osmotic value of the sap rose from 0.036 M to 0.043 M. When however the salt solution was replaced by a solution of salts and mannitol isotonic with the solution of fluoride, the result was the same. The course of the change in the rate of exudation on the addition of NaF (a sudden fall followed by a gradual increase) also demonstrated that an osmotic effect was concerned. So it proved to be necessary to make the solutions without NaF isotonic with those which contained it by adding mannitol. It was impossible to use a Hoagland solution as fluoride precipitated with the Ca present. Instead of an ordinary Hoagland solution one was used in which $Ca(NO_3)_2$ had been replaced by KNO_3 or a solution containing 0.005 M KNO_3 and 0.0005 M KH_2PO_4. It has been demonstrated that the absence of Ca ions does not affect the exudation. Lower concentrations than 0.005 M NaF did not affect the exudation at all, even when they were allowed to act for several hours. Sometimes even when greater amounts of NaF were added no effect could be

TABLE 12

Changes in rate of exudation and osmotic value of the exudation sap caused by the addition of 0.01 M NaF, compared with changes resulting from an enhancement of the osmotic value of the medium (experiment 21).

Medium	time	b	O_b	S
0.005 M KNO_3 (0.013 M) . . .	10.40	9.6	0.036	0.35
0.005 M KNO_3 + M (0.037 M) . . .	11.05	6.0	0.044	0.28
0.005 M KNO_3 + M (0.037 M) . . .	11.30	7.6	0.042	0.32
0.005 M (0.013 M) . . .	12.00	11.4	0.036	0.41
0.005 M + NaF (0.036 M) . . .	12.30	9.0	0.043	0.40
0.005 M + NaF (0.036 M) . . .	13.20	7.4	0.044	0.33
0.005 M + NaF (0.036 M) . . .	13.50	7.4	0.043	0.33
0.005 M (0.013 M) . . .	14.20	10.2	0.037	0.39
0.005 M + M (0.037 M) . . .	14.50	6.0	0.044	0.37

observed. In other experiments the rate of exudation decreased at the addition of NaF. This decrease set in immediately but it was usually rather slight.

The pH of the medium was shown to be very important for the action of NaF. Thus the rate of exudation decreased in an hour to one half, if 0.01 M was added at a pH of 4.3; the same concentration causing no decrease at all at pH 5.2. The change of pH itself did not affect the exudation (c.f. section 3*c*).

Thus the rate of exudation could be inhibited by NaF. The osmotic value of the sap, however, did not change. This means that less salt was given off to the vessels. An inhibition of the whole salt secretion

proved to be concerned: both uptake and tissue secretion were decreased. We could not confirm LUNDEGÅRDH's results, as a decrease of the amount of water given off to the vessels and a simultaneous increase of the osmotic value of the sap were found to result only from the osmotic action of the fluoride solution. It must be pointed out, however, that LUNDEGÅRDH's hypothesis is not the only possible explanation of his results. Similar phenomena would be observed if the salt secretion was not affected but only the water conductivity of the roots (compare the influence of the pH of the outer solution on the exudation). It appears from LUNDEGÅRDH's figures that the inhibition caused by NaF increased with time. This would not happen if the action of NaF was osmotic only for then an equilibrium would be attained after a brief time; but it might be the result of a gradual decrease of k.

The effect of NaF was proved to differ little from that of the other inhibitors mentioned earlier. The only remarkable thing was the great influence of the pH of the medium. LUNDEGÅRDH also found a greater effect at a lower pH, but in his experiments the exudation was already considerably inhibited at pH 5.

e. Iodo acetic acid

The inhibitors discussed up to now appeared to affect both the uptake and tissue exudation. It was to be expected that iodo acetic acid would influence the uptake secretion at least, since it is known that the uptake of salts can be inhibited by this substance. MACHLIS (1944) demonstrated that barley roots did not take up salt if JA was present. LUNDEGÅRDH (1949) found that 0.0002–0.001 M JA at a low pH inhibited the uptake of chloride and nitrate almost completely and decreased the rate of exudation.

Low concentrations such as 0.0001 M were proved not to affect the exudation of tomato plants, at least at a pH of approximately 5. A solution ten times more concentrated influenced the exudation at first no more than the weaker concentration; but after 20–30 minutes the rate of exudation began to decrease gradually. The decrease in the exudation, however, followed a somewhat different course to that observed with the other inhibitors. In their case a decrease was always observed that was rapid at first and slower afterwards (fig. 13, 14) but the rate of exudation now decreased continuously at the same rate so long as the inhibitor was present in the outer solution. The decrease started sooner when the concentration of the inhibitor was higher.

The osmotic value of the sap decreased only when the rate of exudation had decreased considerably; otherwise it remained unaltered. As in the preceding experiments a simultaneous decrease of salt secretion and k would be expected, but in some cases the first seems to decrease to a greater extent.

It appears from fig. 17 that both uptake and tissue secretion were inhibited, but the removal of salts from the medium caused a still further decrease of the exudation even in the presence of JA. This

indicates that the uptake of salt was not fully inhibited. It is difficult to determine to what extent the part of the exudation that depends on the salt uptake had been inhibited, as the decrease of the exudation caused by the lack of salts was enhanced by the prolonged action of JA, in the way that has been described in section 6*a*. After 4½ hours

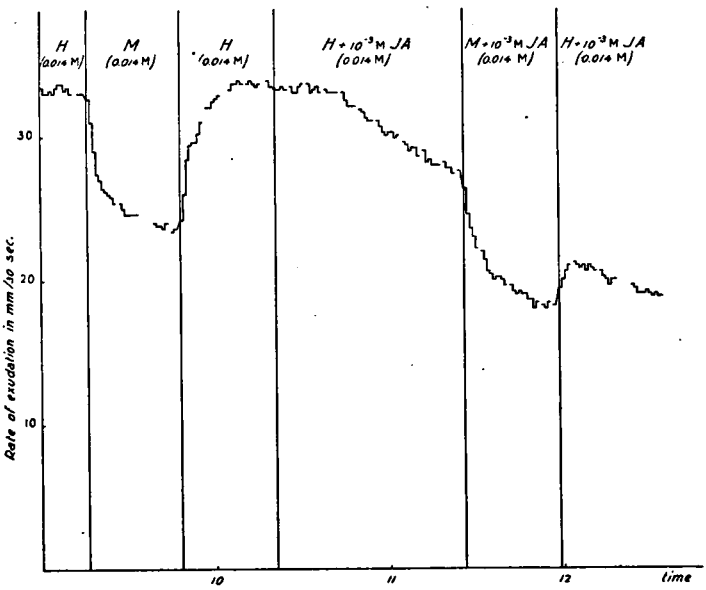

Fig. 17. Inhibition of the rate of exudation under the influence of iodo acetic acid.

no further salt effect occurred. The uptake secretion seems to be completely inhibited at that time, while the tissue secretion is, as yet, not. The rate of exudation did not increase when a solution of JA was replaced by a solution without this substance, but it did not decrease further either. It has been shown by BONNER and THIMANN (1950) that the iodo acetic acid inhibition of the growth of Avena coleoptiles could be overcome by addition of a dicarboxylic acid, malic acid for instance. The inhibition of the exudation, however, did not seem to be affected by the addition of malic acid. No change of exudation occurred if 0.01 M malic acid was added to the outer solution (the pH of the medium had been kept the same, by adding KOH). Neither exudation nor salt secretion increased even when the roots had been on a solution of malic acid for several hours.

f. Phenylurethane

We have up to now described the influence of several substances which are known to act on certain enzymatic processes. Following is an experiment in which the effect of a narcotic, phenylurethane, was investigated. SPEIDEL's experiments (1939) had demonstrated that this substance is able to diminish the exudation of several plants

while HEYL (1930) found that narcotics could affect the exudation of Sanchezia.

With tomato plants the rate of exudation appeared to decrease as soon as phenylurethane (0.001 M) was added to the medium (fig. 18). This decrease followed the same course as in the experiments with KCN, DNP, Na-arsenate and NaF. The decrease in the rate of exudation was accompanied by a decrease of the salt secretion. The exudation on a solution lacking salts proved to diminish after the addition of phenylurethane. As furthermore the removal of salts from the medium resulted in only a small reduction of the exudation when phenylurethane was present, we must conclude that both parts of the exudation had been decreased. The action of phenylurethane corresponded in this respect with that of the other inhibiting substances.

The inhibition proved to be more or less reversible: exudation and

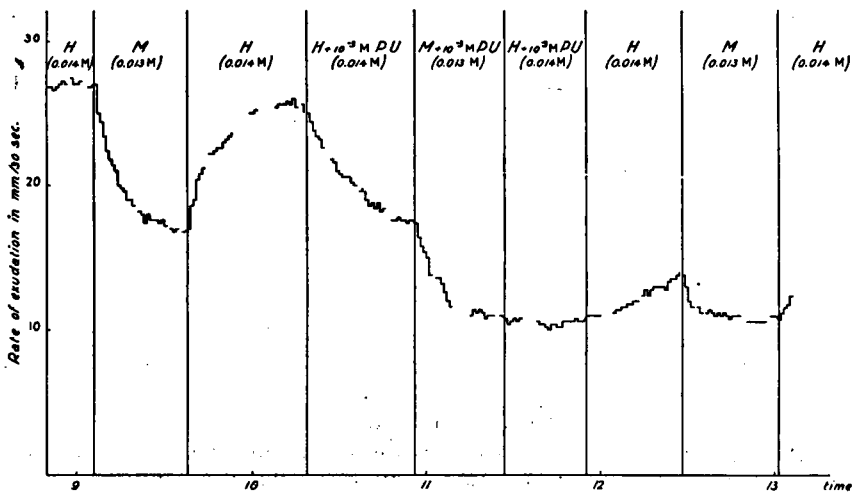

Fig. 18. Influence of phenylurethane on the rate of exudation, in the presence and absence of salts.

salt secretion showed a gradual increase when the solution of phenylurethane was removed. It is remarkable that the recovery of the exudation after removal of phenylurethane seems in the first place to be due to a recovery of the uptake secretion. It appeared that when the salt solution was changed for a solution of mannitol (seventh and eighth period) the rate of exudation decreased to the same level as before in the presence of phenylurethane (fifth and sixth period). This means that the tissue secretion had not yet increased at that time, while the total salt secretion had. The experiment has not been continued, but there is no reason to suppose that it is impossible that the tissue secretion might also increase after a longer time.

Recapitulating we can state that the rate of exudation decreased as the result of adding potassium cyanide, 2,4-dinitrophenol, sodium arsenate, sodium fluoride, iodo acetic acid or phenylurethane to the

medium. Less salt was given off to the vessels, and the water conductivity of the roots decreased; that is to say both water transport and salt transport were affected. The whole salt secretion decreased, but in some cases the transport of salts taken up from the medium was the first to be completely inhibited.

It is remarkable that the exudation reacts so rapidly to the addition of inhibitors (except iodo acetic acid). LUNDEGÅRDH (1949, 1950) also found a rapid effect of KCN: the exudation was already considerably decreased ten minutes after KCN had been added to the medium. He found in his investigation of the inhibition of the cytochrome cytochromeoxidase system by KCN (1952) that the cytochromeoxidase was considerably inhibited in 30 seconds. He concluded on the strength of these results that the cytochromeoxidase is localized at the cell surface. Other substances besides KCN have, however, been proved to act as rapidly, although they are supposed to affect different enzymatic processes. So we must suppose either that all these substances can penetrate the roots very rapidly, or that an important part of the exudation process is localized near the root surface.

The pH of the medium proved to be very important to the action of NaF. The effect of other substances has been investigated at one pH value only, but it is probable that their effect will also be influenced by the pH (c.f. LUNDEGÅRDH 1949). The pH of the medium affects the relation between undissociated molecules and ions, which is known to be important for the action of such substances. The effect is greater as the concentration of undissociated molecules is higher (SIMON and BEEVERS 1952).

From these experiments we can draw only a few conclusions about the way in which these substances affect the exudation process. As the modes of action of various inhibitors are so much alike (JA excepted) it seems plausible to suppose that no specific inhibition of the exudation is concerned, but that the action is more indirect; that is to say that metabolic processes might be affected, which are important for the exudation process. The fact that a narcotic exercises the same influence as substances acting on several enzymatic reactions, also points this way. HOAGLAND and BROYER (1942) stated that the metabolism of decapitated tomato plants was affected by KCN in a similar way as by oxygen deficiency. In both cases less bromide was taken up and the permeability of the tissue decreased. STENLID (1949) thought it possible that the structure of the protoplasm would be changed under the influence of DNP. Such an effect might explain the simultaneous decrease of the transport of salts and water.

In consequence of LUNDEGÅRDH's experiments (1949) in which the exudation was stated to be inhibited by high concentrations and stimulated by lower concentrations of indole acetic acid, the influence of this substance on the exudation of tomato plants was investigated. It seemed on account of the investigations of VON GUTTENBERG and BEYTHIEN (1951) and BRAUNER and HASMAN (1952) that in particular changes of the conductivity for water should be expected although

we must bear in mind that in these experiments the water permeability of tissue was investigated and not that of intact roots. SKOOG, BROYER and GROSSENBACHER (1938) found a stimulation of the exudation of Helianthus, if indole acetic acid was applied to the stem. More salt was given off to the vessels in this case.

Like LUNDEGÅRDH we added indole acetic acid to the outer solution. The influence of indole acetic acid on the exudation proved on the whole to be very small. A stimulation could not be ascertained with sufficient certainty. Higher concentrations (1–10 mg/L) caused a decrease in the rate of exudation, but k was hardly changed. The osmotic value of the sap decreased somewhat so that the decrease in the rate of exudation must be attributed chiefly to a decrease of the salt secretion. This inhibition occurred later than in the experiments with inhibitors described earlier, and was not so strong.

§ 7. PERIODICITY

Authors like HOFMEISTER (1862) and WIELER (1893) have already demonstrated that the rate of exudation of various plants shows fluctuations during the day, so that the greatest amount of sap is given off approximately at noon while a minimum occurs in the night. HEYL (1930) attributed this phenomenon to fluctuations of temperature during the experiments. However, it was also observed by others when the temperature was kept constant (HOFMEISTER; SPERLICH and HAMPEL, 1936). GROSSENBACHER (1939) stated that, with Helianthus, periodicity could be influenced by several circumstances: The exudation of plants grown under normal conditions, showed a maximum by day and a minimum during the night, whereas the reverse happened when the plants had been kept in darkness during the day and had been illuminated overnight. This seemed to indicate that the periodicity of exudation was caused by the alternation of periods of light and darkness during growth, as SPERLICH and HAMPEL thought. However, periodicity also occurred, with plants that had been grown in constant light, or in permanent darkness (that is in weak red light); the moment at which maxima and minima occurred now depending on the time of decapitation. Changes of temperature could also exercise some influence.

SPEIDEL (1939) also found a relation between the moment at which the plants had been decapitated and the time at which the maxima and minima were observed, when investigating the exudation of several plants (Helianthus, Ricinus and others). The nature of the medium was also of importance: plants grown in sand showed their first maximum approximately 12 hours after decapitation (this period seems rather variable), plants in a nutrient solution 12 hours later. The maxima could be shifted by a rise in temperature. HEIMANN (1952) came to a similar conclusion: the time of decapitation was of importance for the time at which the maximal exudation could be observed with Kalanchoe Bloszfeldiana. The rhythm could be influenced by illumination; light having a stronger influence as more leaves were left on the exuding stump.

According to WHITE (1938) the phenomenon of periodicity should be independent of external conditions, as isolated tomato roots, that had been cultivated for five years, showed a diurnal fluctuation of root pressure. Changes of temperature were considered unimportant, while the influence of light might in this case be left out of consideration.

A diurnal periodicity has been demonstrated not only in exudation but also in guttation. The cycles of guttation could be influenced by changes in the periods of illumination (ENGEL and FRIEDRICHSEN, 1951, 1952; HEIMANN 1950). It appears from this, that such a rhythm also occurs in intact plants so that it cannot be considered a result of decapitation (c.f. SPEIDEL).

Changes of permeability have repeatedly been supposed to be involved with the periodicity of exudation (GROSSENBACHER 1938; SKOOG, BROYER and GROSSENBACHER 1938; ENGEL and FRIEDRICHSEN 1952), but it seems that no investigation has ever been made to find out whether the permeability does actually change. SKOOG, BROYER and GROSSENBACHER found that the amounts of salt present in the sap of Helianthus annuus also fluctuated, while the periodicity could be intensified by applying indole acetic acid to the stem.

When describing the experiments with tomato plants we pointed out repeatedly that irregularities in the exudation might be the result of periodic changes in the rate of exudation. The observation of such irregularities was, in connection with the data from the literature just mentioned, a reason for investigating the course of the exudation rate, salt secretion and k over several successive days. In table 13 the results are given of an experiment that has been carried out in the following manner: rate of exudation, osmotic value of the sap and k were determined every hour in precisely the same way that has been described in section 6 (experiment 17*a*). The nutrient solution was renewed every two hours in these experiments to avoid important changes of the salt concentration of the medium.

We see in experiment 24 that the rate of exudation was 9.9 mm/30 sec at 7 a.m.; it increased to 21.1 at 11 and afterwards decreased to 7.7 mm/30 sec at 7 p.m. On the second day the rate of exudation was much slower than on the first, but it proved to have increased when compared with the last observation of the previous night. During the night no observations were made, but the sap was collected during that time, so that the average rate of exudation could be calculated. This amounted to 3.2 mm/30 sec, which was less than both the last value observed in the evening and the first of the next morning. This indicates that still a lower value must have been attained during the night. So the exudation of tomato plants was actually proved to show a maximum by day and a minimum at night. The second day a maximum was found once more between 11 and 14 hours; this cannot be seen in experiment 24 as the circumstances were changed at 11 o'clock.

The osmotic value of the sap proved to change very little during the day. A small decrease occurred by the end of the afternoon. No further important changes could be observed the second day. It

TABLE 13

Periodic changes of rate of exudation and conductivity for water under various conditions.
* means that sucrose has been added to the medium

time	24 Tomato Hoagland solution			24*a* Tomato distilled water			24*b* Tomato Hoagland solution containing 0.02 M sucrose			24*c* Tomato Hoagland solution leaves in sucrose			24*d* Tomato Hoagland solution. Decapitated at 17.30	8.30	24*e* Sanchezia nobilis. Hoagland solution		
	b	O_b	*k*	*b*	O_b	*k*	*b*	O_b	*k*	*b*	O_b	*k*	*b*	*b*	*b*	O_b	*k*
7	9.9	0.032	387	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	14	0.032	568	11.1	—	422	22.9	0.040	950	10.0	0.048	258	11.0	—	—	—	—
9		0.032	615	11.0	0.026	403	29.7	0.044	1119	13.0	0.047	329	12.0	2.2	—	—	—
10	16.4	0.030	694	16.3	0.022	553	32.7	0.050	945	15.5	0.046	396	12.4	3.5	—	—	—
11	20.5	0.031	742	14.7	0.022	561	32.9	0.047	906	17.9	0.049	454	12.6	4.4	25.0	0.028	706
12	27.1	0.031	658	12.2	0.018	554	34.6	0.044	869	19.0	0.045	471	12.6	5.0	26.0	0.028	740
13	17.0	0.031	584	11.0	0.018	483	33.2	0.045	825	18.7	0.041	446	12.9	5.0	26.5	0.027	778
14	14.5	0.029	580	11.3	0.016	411	32.6	0.051	800	16.1	0.043	379	12.0	5.6	26.5	0.027	765
15	13.5	0.029	516	8.7	0.016	350	31.3	0.045	806	14.0	0.044	358	12.0	6.3	24.0	0.028	669
16	12.5	0.032	503	8.5	0.018	353	29.0	0.047	737	11.9	0.043	318	11.4	6.3	23.0	0.027	612
17	11.1	0.029	484	6.8	0.019	355	21.9	0.045	604	9.8	0.044	296	11.8	6.3	—	—	—
18	9.0	0.027	436	5.0	0.017	280	18.5	0.051	500	8.1	0.045	238	11.0	6.6	—	—	—
19	7.7	0.026	336	3.6	0.015	217	12.2	0.045	313	5.1	0.043	175	—	6.6	—	—	—
20	6.0	0.025	274	2.4	0.018	148	9.6	0.044	252	4.0	0.042	139	—	—	—	—	—
21	4.9	0.026	242	2.6	0.015	—	—	—	—	—	—	—	—	—	—	—	—
22	4.2	0.027	219	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22–7	3.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	3.9	0.022	213	—	0.011	—	3.7	0.046	148	3.0	0.039	100	—	—	24.5	0.029	761
8	3.9	0.021	200	3.4	0.010	479	4.0	0.048	154	3.9	0.022	100	—	—	24.9	0.029	725
9	4.4	0.019	226	4.4	0.011	513	4.5	0.046	185	4.5	0.039	117	15.2	15.7	25.4	0.026	692
10	5.0	0.022	232	5.6	0.013	547	5.3	0.044	177	6.3	0.041	127	17.0	16.6	25.4	—	708
11	6.0	0.024	245	7.5	0.013	563	5.5	0.045	138	6.7	0.040	137	18.4	17.7	24.4	0.031	704
12	8.5*	0.024*	254*	8.8	0.011	547	5.5	0.044	123	5.9	0.040	118	19.2	19.3	24.2	0.030	704
13	15.0*	0.031*	250*	9.8	0.013	528	—	—	—	—	—	—	18.8	19.5	23.8	0.032	700
14	11.5*	0.033*	209*	—	—	—	—	—	—	—	—	—	18.8	18.8	20.2	0.033	554
15	—	—	—	—	—	—	—	—	—	—	—	—	16.6	17.–	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	15.2	16.2	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	12.6	14.2	—	—	—

follows from this first that a maximal quantity of salt was given off at the time when the rate of exudation was largest (table 13) and secondly that k must have changed too. Indeed k proved to increase from 387 at 7 a.m. to 658 at 11 a.m. and to decrease afterwards. At 7 a.m. the value of k amounted to 336.

This experiment was carried out with a plant that had been grown under normal conditions of illumination. Corresponding results were obtained with all such plants, although individual differences might occur, so that the changes were sometimes less distinct. In the same table data are recorded of an experiment that had been conducted in the same way; the medium however, consisted not of a Hoagland solution but of distilled water. Exudation, salt secretion and k showed a maximum between 10 and 12 a.m., just as in the former experiment. The changes in magnitude of these three ran parallel as in the other case. The second day however, the rate of exudation had much decreased and less salt was given off, but k was as large as on the previous morning. This proved to be a typical difference between plants on a salt solution and those on distilled water.

Thus we have seen that the periodic changes in the rate of exudation of tomato plants are accompanied by changes in the amount of salts given off to the vessels and in the conductivity for water of the roots. The salt secretion appeared to have greatly decreased one day after decapitation. Depletion of salts in the roots might be the cause when the plants had been on distilled water and addition of salts to the medium had a favourable effect.

However the fact that just the same happened with plants that could take up salts, indicates that others factors must be involved. It seemed probable that a decrease of the metabolic activity caused by depletion of substances in the roots would be concerned. It has been shown that the exudation could be promoted by the addition of sugar to the medium (fig. 11). It therefore appeared plausible to suppose, that deficiency of sugar would be the cause of this decrease. It appears from table 13 (experiment 24) that exudation and salt secretion increased when sucrose was administered to a plant that was still exuding weakly, even though the addition of sucrose caused an increase in the osmotic value of the medium. This stimulation, however, was only temporary: after about one hour the exudation decreased again, and less salt was given off. When a plant was put on a salt solution containing 0.02 M sucrose immediately after decapitation, a considerable exudation could be observed over two days, the course of which was just the same as when no sucrose was administered (experiment 24*b*). Periodic fluctuations in exudation, salt secretion and k also occurred when the plants were decapitated right over the two lowest leaves, so that these could be submerged in a sucrose solution (experiment 24*c*). Thus we did not succeed in demonstrating any influence of the sugar supply on periodicity, which corresponds with WENT's results (1944).

The experiments described up to now were carried out with plants that had been in the light by day and in darkness during the night.

In later experiments however plants were used, that had been illuminated overnight, while they were kept in the dark during the day. We see from experiment 24*d* (table 13) that these plants also show a maximal exudation, salt secretion and conductivity for water by the end of the morning. So in contrast to GROSSENBACHER (1939) we find no influence of the period of lighting, except if it might be that the rhythm is fixed at an early stage of development; for as we have said, the plants were in an ordinary green-house during the first 4–6 weeks; after that they were kept in artificial light at night for 5–6 weeks.

The diurnal fluctuations of these plants were usually much smaller. In particular the exudation showed strikingly small changes during the first day after decapitation. Only in the afternoon did the exudation begin to decrease. A distinct maximum could not be observed, although the average exudation was stronger by day than by night. Next day however, the fluctuations were more obvious. This might indicate that the periodical changes are not a normal phenomenon, but that they are somehow caused by decapitation or one of its after-effects. It was necessary now to investigate whether perhaps the moment of decapitation might determine the time at which the maxima occurred. The plants were usually decapitated the evening before the experiment was to take place. When they were cut off at 8 a.m., however, the exudation proved to increase gradually during the first day (table 13, experiment 24*d*); next day, that is a good 24 hours after decapitation, they showed a maximal exudation at precisely the same time as plants that had been decapitated 15 hours earlier. So we could not confirm the results of SPEIDEL, HEIMANN and GROSSENBACHER.

We have already pointed out that the tissue exudation at any rate shows periodic fluctuations (experiment 24*a*). It may be questioned whether the same holds true for the part of the exudation that depends on the uptake of salts. This was investigated by determining the salt effect at various times of the day. For this purpose the plant was transferred from a Hoagland solution to a solution of mannitol. The solutions were changed again after half an hour. However, it proved to be difficult to gain a proper insight into the changes of the uptake secretion. This must be determined by subtracting the amount of salt given off per unit of time on a solution lacking salts from the quantity that is given off when salts are present in the medium. These values cannot be determined at the same time. When as in this case the tissue secretion is very variable, incorrect values must be obtained for the uptake secretion. For this reason the uptake secretion was calculated in the following way: we determined the total quantity of salts given off immediately before the substitution of a mannitol solution and half an hour after the mannitol solution had been changed again for a Hoagland solution. From those data we estimated the value that the total salt secretion would have had at the moment at which the tissue secretion was determined. However this method is not very accurate either. The data obtained in this way are given

in table 14. They indicate that the uptake secretion also changes, but the changes are not very evident.

The composition of the sap proved to change little during the day (table 6). This corresponds with the fact that the osmotic value of the sap was not very variable. The amount of phosphate given off changes comparatively more than that of nitrate, but we must consider

TABLE 14
Periodic changes of the salt secretion

Experiment	time	amount of salt given off on a Hoagland solution	amount of salt given off on a mannitol solution (tissue secretion)	uptake secretion
25	9.15	0.68		
	9.50		0.55	0.15
	10.25	0.71		
	11.55	0.65		
	12.30		0.41	0.19
	13.05	0.54		
	14.25	0.52		
	15.00		0.32	0.15
	15.35	0.41		
	16.40	0.35		
	17.15		0.20	0.08
	17.50	0.21		
25*a*	9.20	0.49		
	9.50		0.35	0.18
	10.20	0.57		
	12.00	0.63		
	12.30		0.42	0.20
	13.00	0.61		
	14.30	0.63		
	15.00		0.37	0.25
	15.30	0.61		
	16.45	0.48		
	17.15		0.24	0.18
	17.45	0.35		

the possibility that in this case the error of determination might have some influence since the concentration of phosphate was sometimes very small.

The exudation of Sanchezia nobilis proved to be much more regular over a longer time. This has already been seen in table 6 (experiment 10*a*): both quantity of sap and of salts given off per unit of time changed very little over 24 hours. In some cases greater fluctuations were observed. In experiment 24*e* (table 13) the greatest rate observed was 26.5 mm/30 sec, the smallest 23 mm/30 sec. K decreased simultaneously from 778 to 622. These changes are much smaller than those observed with tomato plants (table 14). Rate of exudation, salt secretion and k were proved to change to approximately the same extent.

These plants were grown under the same conditions as the tomato

plants, that is in the lighted green-house. According to HEYL diurnal fluctuations in the exudation of Sanchezia can be attributed to changes in the temperature of the surroundings. This could not be the case here; even though the temperature of the room might change a little; for the temperature was not at a maximum at noon, but at the end of the afternoon, a time when the exudation was already decreasing again. Moreover changes in air temperature of only $\pm$ 2° C were concerned. So we have not succeeded in finding a relation between some external condition and periodicity. A more extensive investigation would, however, be required to decide whether the periodicity of these plants cannot in fact be influenced by external conditions.

IV. GENERAL DISCUSSION

When planning this investigation we started from the working hypothesis that the exudation process would consist of an active transport of salts to the vessels and an osmotic transport of water caused by the resulting difference between the osmotic values of the xylem sap and the outer solution. If this is true two internal factors should control the exudation: the secretion of salts into the vessels and the water conductivity of the roots. The latter factor, naturally, influences the transport of water and in that way the concentration of the sap in the vessels.

We will first discuss these factors separately in the light of our experimental results and afterwards we will try to give a picture of the exudation process as a whole.

Salt secretion

In section 1 we have argued that the exudation might consist of two parts: that caused by salts given off from the tissue and at the same time the additional transport of water caused by salts taken up from the medium and given off to the vessels. These conclusions were drawn from the following facts: the course of the change in the rate of exudation indicated that less salt is given off to the vessels immediately the uptake of salts is inhibited, but nevertheless that exudation and salt secretion may continue at a slower rate for a long time. This part of the exudation and secretion could be influenced by changes in the osmotic value of the medium, while so far as the exudation resulting from salt uptake was concerned, only the transport of water proved to be so dependent, the transport of salts being influenced only by the salt concentration of the outer solution.

We will first be concerned with the case when no salt is taken up from the medium, so that only the tissue secretion is active. It will be remembered that the expression tissue secretion is used for the whole process concerned with the transfer of salts from the tissue into the vessels.

It is known that salts can be carried from the roots to the shoot

when plants are on distilled water (STEWARD, HARRISON and PREVOT, 1942; HOAGLAND 1948). The salt content of the tissue is much decreased in such a case. The plasm is usually supposed to contain few free ions (ARISZ 1945). As it seems improbable that ions like nitrate and chloride would be present in large quantities in a bound state, we must assume that ions will be given off from the vacuole. It is possible that with phosphate the situation is different. HYLMÖ (1953) concluded that in the roots of intact pea plants calcium would leak from the vacuole and be transported to the shoot, when the medium consisted of distilled water.

Little is known of the way in which salts are given off from the vacuole. Salts, that have been accumulated in the vacuole are generally supposed to be given off with some difficulty (HOAGLAND 1948). It seems probable that the rate at which these salts are given off will control the magnitude of the tissue secretion.

We found a decrease in the exflux of salts from the tissue when the osmotic pressure of the medium was enhanced. The reason for a relation between the osmotic value of the medium and the rate at which salts are given off from the cells, is not very obvious. LUNDEGÅRDH (1945) gave the following explanation: when salts are given off by a cell, its osmotic value decreases, so that water is exuded at the same time. This exudation of water from the cells is determined by their turgor pressure, which depends in its turn on the osmotic pressure of the medium. A smaller quantity of this salt solution is pressed out when the turgor pressure is less. It seems plausible to seek the answer to this problem along these lines, that is by supposing that a salt solution is given off from the tissue. It must be understood however, that there remain some differences between our view and that of LUNDEGÅRDH. It has been demonstrated in the experiments of ARISZ, HELDER and VAN NIE (1950, 1951) as well as in our own, that the water movement is osmotically regulated even when no salts are present in the medium. The amount of sap exuded decreases, while its osmotic value increases as the osmotic value of the medium is enhanced. This regulation is very rapid. This indicates that if a salt solution is given off from the tissue, more water must be sucked in from the medium as a result of the concentration difference between this salt solution and the outer solution. LUNDEGÅRDH did find a decrease in the rate of exudation but no change in the concentration of the sap. For this reason he concluded that the solution was given off in a smaller quantity, but that the relation between salt and water was unchanged.

LUNDEGÅRDH supposed that only the vascular epithelium would exude a salt solution. It seems however more plausible to suppose that salts are given off from the whole root tissue. It has been shown in the experiments with inhibitors that the exudation was quickly decreased by a considerable amount, but that it took a much longer time before the tissue secretion had been fully inhibited. This could be considered as an indication that regions towards the periphery of the roots as well as the more central parts are of importance for the salt secretion. The fact that the tissue secretion is fully inhibited later

than the uptake secretion might also be explained in this way. It seems questionable, however, if this can be considered as sufficient evidence, since it is known that various inhibitors can permeate very rapidly.

There are few data on the way in which salts are transferred from the tissue to the vessels. The theories that have been advanced on this subject are mostly concerned with the case when salt is taken up from the medium. They will be discussed below.

We supposed that salts after having been taken up from the outer solution, could be transported directly to the vessels. This supposition is not a new one. HOAGLAND (1940) has already pointed out its possibility. BROYER (1950) found in his investigation on the uptake of radioactive bromide by high salt roots, that the exudation sap contained more radioactive bromide than the sap expressed from the roots. From this he concluded that in such circumstances bromide might migrate to the vessels through the cytoplasm circumventing the vacuole. ARISZ (1948, 1952) also came to the conclusion that in the cells of leaves of Vallisneria spiralis chloride ions could be transported through the plasm without having been first accumulated in the vacuole. This process was not affected by KCN, nor by DNP.

We found that the exudation — and thus the salt secretion into the vessels — increased within a few minutes, if a solution lacking salts was replaced by a solution of KCl or KNO_3. It may be questioned whether this means that within that time enough ions taken up from the medium have reached the vessels to cause an apparent increase in the exudation. It appeared from determinations of the composition of the sap, that in similar cases the increase of the exudation must be attributed to a secretion of chloride or an increased secretion of nitrate. It is not possible however, to put this fact beyond dispute. It might be that the increase in the rate of exudation is due at first to a larger secretion of ions already present in the root. It has generally been found, that the transfer of salts across the roots takes rather more time. CRAFTS and BROYER (1938) found bromide in the exudation sap of squash plants half an hour after it had been added to the medium. As the transport of salts from the roots to the cut surface would take approximately 10 minutes according to their calculation, 20 minutes would be required to bring salt from the medium into the vessels. LUNDEGÅRDH (1949) found that changes in the salt concentration of the medium became evident in the sap only after a quarter of an hour. In his experiments on uptake and transport of neutral red he stated that the dye was first accumulated in the vacuoles of the cortical cells. Afterwards it disappeared, and it could be found in the walls of the vessels within half an hour. In LUNDEGÅRDH's opinion this would indicate the way in which ions are transported through the roots. The fact that the dye was first accumulated in the vacuoles indicates that the tissue secretion was concerned here. We pointed out in section 3*b* that there are indications that not all ions are transported to the vessels in the same way. It follows from this that it is not

possible to apply uncritically data on the transport of a certain substance to the transport of all ions.

Part of the salt taken up must go to the xylem vessels. It has, however, been shown that accumulation in the tissue can take place at the same time. HOAGLAND (1948) found the same. It may therefore be asked whether in the case of salt uptake from the medium salts can be accumulated in and given off from the vacuole at the same time. It might also be possible, that in such circumstances all nitrate given off to the vessels comes from the medium. The tissue secretion would then only begin when the supply of salts from the outer solution was cut off. It is difficult to decide this question. It has been shown by determinations of the salt content of the sap that nitrate and chloride can be given off at the same time, while only chloride is taken up. This is, however, no proof that the same ion can at the same time be transported directly to the vessels and also given off from the cells. There is however a more indirect indication that this is actually true. We have demonstrated that only the tissue secretion decreases when the osmotic pressure of the medium is enhanced. The fact that less salt is given off to the vessels when the osmotic value of a salt solution is enhanced (table 2 and experiment 16) indicates that the tissue secretion must be active. The decrease of the salt secretion may be so great that a decrease in the secretion must be involved, since the other salts are quantitatively unimportant.

Both uptake and tissue secretion were shown to decrease under the influence of inhibitors. This suggests that these processes coincide at least partially. Where they do so could be in the transport of salts through the symplast of the roots to the vessels.

Administering sugar proved to promote the exudation only when salts were present in the outer solution. We mentioned the possibility that sugar promotes the uptake of salts in the symplast. It proved however, to take some time before the effect of sugar was perceptible. This might indicate that sucrose must be taken up into the tissue before it can act on the exudation. In that case a promotion of the transport should rather be expected. It therefore remains remarkable that the tissue exudation was not affected when the outer solution did not contain salts, but it might be that in this case the rate at which salts are given off by the tissue controls the salt secretion.

LUNDEGÅRDH (1950, c.f. BURSTRÖM 1951) advanced a theory on the transport of salts to the vacuole and to the vessels. According to his idea anions should be transported across the cytoplasm along a bridge of cytochromesystems. Oxidation should take place at one end of the bridge (the cell surface), reduction at the other (vacuole or vessels), so that anion respiration would be of importance. WEEKS and ROBERTSON (1950) developed a similar idea.

The inhibition of salt secretion by KCN would be consistent with the supposition that the cytochromesystem is concerned with the transport of salts. However according to the theory just mentioned transport of ions depends on the anion respiration, which operates only if salts are present in the medium. In our experiments a notable amount

of salt proved to be transported to the vessels when salts were lacking in the outer solution. This transport was also affected by KCN. We have pointed out already that it seems more probable that the inhibitors act more indirectly on the exudation.

LUNDEGÅRDH's theory cannot account for the differences in the transport of the various ions (nitrate and chloride on the one hand, phosphate on the other). HELDER (1952) thinks that ions may be bound to a certain carrier, perhaps in connection with metabolism (c.f. STEINBACH). He supposes, however, that transport might take place by the cytochromesystem, when the salts have entered the plasm. It remains questionable if this can explain the differences observed here.

Phosphate seems to be retained in the tissue to a greater extent than nitrate and chloride, as is indicated by the fact that it takes so much time before phosphate that has been taken up reaches the vessels. This is consistent with the supposition that phosphate will rapidly be bound chemically in the protoplasm. ARISZ (1952) even points to the possibility that phosphate might be transported in a bound state.

We have mentioned the view of CRAFTS and BROYER concerning the process of salt transport and exudation. WIERSUM (1947) thinks that cells within the endodermis have as the result of lack of oxygen a lower accumulation level than the cells at the surface of the roots, so that leakage of salts to the vessels should occur. ARNOLD (1952) like LUNDEGÅRDH believing that each cell takes up salt and gives it off, so that there is no transport through the symplast, thinks that salts are given off from the cells by an active process, controlled by glycolysis. This opinion is founded on a hypothesis of LUNDEGÅRDH (1949). LUNDEGÅRDH himself revoked this supposition (1950). According to his opinion salts are exuded passively from the cells. This process is not connected with metabolism, but it can occur rapidly because of the high salt permeability of the tissue. By this is meant a kind of exchange at the cellular interfaces, which might at the same time explain why only salts, and not organic substances, are given off to the vessels. HOAGLAND (1948, c.f. BIDDULPH 1951) calls the process that controls the exflux of salts into the vessels "a process akin to secretion". ALBERDA (1948) also thinks that a secretion process is concerned: the salt deficiency caused by a secretion of salts into the vessels would cause the transport of salts in that direction. ARISZ, HELDER and VAN NIE compare this process with the secretion of salt by the salt glands of Statice. ARISZ (1953) succeeded in demonstrating that the accumulation of chloride in the vacuole of the leaf cells of Vallisneria was inhibited by DNP, but the uptake from the medium by KCN, while the transport through the protoplasm was affected in neither case. This implies that different active processes are concerned with uptake into the plasm and secretion into the vacuole, but we do not know if the same holds true for the secretion into the vessels. Considering the transpiration stream as a passive mass flow from medium to shoot through cell walls and cytoplasm

HYLMÖ (1953) gives the following working hypothesis of the exudation process: he supposes that with the development of the interconnecting vessel elements in the xylem the plasm surfaces continue to function for some time when the transverse upper wall has been ruptured. This xylem cell would accumulate ions in the same way as the other cells of the root tissue. The plasm, probably the tonoplast, functions as a semipermeable membrane. Thus there is no secretion into the xylem but the xylem elements take up ions, which causes a passive flow of water.

From our own experiments we can only conclude that it seems less probable that there is a leakage of salts to the vessels along a concentration gradient, since it would then be difficult to explain the sudden large decrease or increase of the salt secretion when salts are removed from or added to the medium, because in such circumstances some after-effect would be expected. Whether there is a secretion or an accumulation of ions into the xylem vessels we cannot yet decide.

Water conductivity

It has already been stated in the Introduction that k in the formula $b = k(O_b - O_m)$ should be considered a measure of the water conductivity of the roots. The value of k depends on several circumstances. It reflects both the size of the root system and its resistance to water transport.

K has been shown to change in several cases. This might happen under constant conditions, a case which will be discussed later. Changes of k could also be observed as the result of a change in the osmotic value or the pH of the medium, and under the influence of inhibitors. The changes were usually reversible, only inhibitors could sometimes cause an irreversible decrease of k.

A decrease of k appeared to be nearly always accompanied by a diminished exudation as well as by a reduced salt secretion. The reverse also held true.

Several authors mentioned the possibility that the resistance of the roots to water might decrease as more water was sucked through them. JOST (1916) made this supposition because it appeared from his experiments that the suction tension in the vessels resulting from the transpiration of the shoot would otherwise be improbably high. KÖHNLEIN (1930) while criticizing JOST's methods, came to the same conclusion in a different way. BREWIG (1937, 1939) demonstrated that the apical zones of roots of Vicia Faba were more permeable to water than the basal parts, but the resistance of the basal parts appeared to decrease more if the suction tension in the vessels increased. These results were confirmed by BROUWER (1953). LUNDEGÅRDH (1945) found that the quantity of water sucked through wheat roots did not increase proportionately to the suction force but to a greater extent. At first he supposed valves to be present which would open at a certain pressure, but later (1950) he stated that the cortex would let pass much more water when the intercellular channels did not contain air any more but were filled with water. It would take

some time before all air would be gone; from that instant, however, water could pass more easily.

These decreases in the resistance to water flow which mean an increase in the water conductivity of the roots, were always accompanied by an increase of the suction tension in the vessels. (BREWIG, BROUWER). In our experiments rate of exudation and water conductivity increased but there was no rise of the suction tension. The suction tension in the vessels even decreased when the osmotic value of the medium was lowered, while k nevertheless increased. When k changed owing to periodicity or the effect of inhibitors the suction tension remained unaltered. This indicates that in spite of the apparent similarity we have been examining a different phenomenon to that discussed by the authors quoted above.

The fact that changes of salt secretion and k run parallel might indicate that these two are interdependent. This would be so, for instance, if salts and water were given off together to the vessels by one process which could be influenced by internal and external conditions. In that case it would not be possible to take k as a measure of the water conductivity. We have, however, seen that under certain conditions, viz. when there is a reduction of the salt secretion as the result of a decrease in the salt concentration of the medium k remains unaltered, or even increases (when a Hoagland solution is replaced by distilled water). Conversely it appears possible to diminish k by lowering the pH of the medium without the salt secretion changing considerably. From this we can conclude that the salt secretion and k are not necessarily interdependent. The cause of their simultaneous change is to be sought in a change of a process that influences both or that both possess in common (such as transport through the cells). In section 6 we have already concluded that inhibitors might affect both salt secretion and water conductivity by acting on metabolic processes important to the state of the protoplasm, for which there is a parallel in the phenomenon concerned with oxygen deficiency (HOAGLAND and BROYER 1942; BROYER 1951). The last case of simultaneous changes of k and salt secretion resulting from changes in the osmotic pressure of the medium is less clear. We have seen that if we follow LUNDEGÅRDH's idea the decrease of the salt secretion would be connected with a decrease of the turgor pressure of the tissue. BROUWER's experiments, however, would indicate that in such circumstances the conductivity for water should rather show an increase. It may be observed, however, that the changes of turgor pressure in BROUWER's experiments must have been much greater than in ours.

We mentioned above that the resistance to the movement of water decreases when a solution of salts is replaced by distilled water. ARISZ, HELDER and VAN NIE considered this a specific effect of the salts present in the Hoagland solution. It appears however from the experiments given in section 1, that k does not change if a Hoagland solution is replaced by another solution lacking salts but isotonic with it. The increase of k in the former case must result from the simultaneous decrease in the osmotic value of the medium. We did not

succeed in demonstrating any specific effect of ions on k. Other authors (ROSENE 1941; HAYWARD and SPURR 1943; LONG 1943) have not found any influence on the uptake of water by roots either.

As to the question of which way water moves to the vessels we may observe that the influence of inhibitors on the water conductivity points to the plasm being involved. This is confirmed by the fact that changes in the pH of the medium can also affect k. We cannot, however, decide whether only the protoplasm of the endodermis is concerned or that of the other cells of the roots as well.

We have given the following conception of the exudation process: a solution of salts flows from the root cells into the vessels. This solution attracts water from the medium osmotically. In addition salt taken up from the medium can be given off to the vessels. This also results in an osmotic water transport.

There is an objection to this idea concerned with the determinations of the osmotic values of the exudation sap. ARISZ, HELDER and VAN NIE stated that these values were usually lower than should be expected on the strength of the formula $b = k\ (O_b - O_m)$. We repeatedly found the same (table 2).

ARISZ, HELDER and VAN NIE mentioned various explanations for this phenomenon. In the first place experimental errors might be involved. We have discussed this possibility before. It seems rather improbable that experimental errors would be important on account of the accurate method which was used for determining the osmotic value of the exudation sap. Setting aside this possibility it might be supposed that salts are taken up from the sap in the vessels during the upward transport. In the second place it must be kept in mind that the formula just mentioned was derived starting from the supposition that the zone of the roots in which salts are given off to the vessels coincides with the water absorbing zone. If the latter is larger, the sap will be diluted as more water will be sucked in afterwards. Finally a similar deviation would be observed if water and salts were given off together.

In chapter III section 1 we have demonstrated that the osmotic value of the sap may also be higher than we should expect from the exudation observed. This might indicate that water is taken up from the sap during the upward transport. This seems rather improbable. Another explanation would be that the surrounding cells secrete salt or a concentrated salt solution into the vessels. This must happen then in the zones of the roots where osmoregulation is difficult, that is in older parts. Our difficulty is that we have to explain two conflicting facts. In both cases we pointed out that salts might be given off as a solution. It is known for instance that leaves of salt plants also secrete salt as a solution. We have already pointed to the probability of a simultaneous transport of salt and water by the process we called tissue exudation.

It is known that in excised roots most salts are accumulated in the

apical zone (PREVOT and STEWARD 1936; STEWARD, PREVOT and HARRISON 1942). STEWARD, PREVOT and Harrison compared the accumulation of salt in excised barley roots and in the roots of intact plants. They found that in both cases most salt was accumulated in the apical zone, but much more in the first case than in the latter. This difference was greater than that in the accumulation in the basal parts in both cases. From this they concluded that the greatest quantity of salt would be given off to the vessels in the apical zone. The experiments of BREWIG and BROUWER demonstrated that the resistance to water transport is smallest in the tip zones. It is probable that the exudation is mainly localized in this part. As ARISZ, HELDER and VAN NIE already pointed out, we do not know if the zone in which most salt is given off coincides with the zone of the smallest resistance. The uptake secretion may be localized chiefly in the apical zone, but it is possible that the tissue secretion covers a larger zone, as it seems probable that the older parts of the roots give off salt accumulated before. LUNDEGÅRDH (1950) investigating the exudation of root pieces distinguished a tip zone of approximately 30 mm and a basal piece of 50 mm. The latter was divided into an upper and a lower part. The upper part of the basal piece was proved to give off more nitrate than the other parts and the exudation sap was more concentrated. This agrees with our supposition that the older parts of the roots also give off salts, while the water movement is hampered because of the higher resistance of the root. We may observe that it seems possible that in the conditions of LUNDEGÅRDH's experiments the tissue secretion predominated, as very young plants were used. This might explain some of the contrasts between LUNDEGÅRDH's experiments and our own. BROYER (1950) found a direct transport of salts to the vessels only with high salt plants. In our experiments also plants were used that had been grown under such conditions that the roots must have been in a rather high salt condition. It is not impossible that with low salt roots accumulation into the vacuole might prevail, so that little salt is available for direct transport to the vessels. The exudation of low salt plants is so weak that it is difficult to perform any experiments with them.

To gain a more thorough understanding of the exudation process it will be necessary in the first place to collect more data on the localization of the various processes concerned.

Periodicity

The periodic fluctuations of the rate of exudation proved to be accompanied by changes in the amount of salt given off and the water conductivity. Various authors had already suggested the latter possibility (SKOOG, BROYER and GROSSENBACHER 1938; GROSSENBACHER 1938). The periodic cycles in the transpiration of plants showing a maximum by day and a minimum during the night under constant conditions (MONTERMOSO and DAVIS 1942) might also be explained by assuming changes in water conductivity. The investigations of SKOOG, BROYER and GROSSENBACHER showed that more factors must

be involved. They also found fluctuations in salt secretion. The maximum occurred a little later than that of the exudation, while in our experiments both occurred at the same time.

We have ascertained that the tissue secretion at least shows a periodic fluctuation. This was, however, less obvious for the uptake exudation. If changes in the tissue secretion only are concerned, it would be attractive to connect this phenomenon with another case of a simultaneous change of tissue secretion and conductivity for water, that occurs when the osmotic value of the medium is changed. Above we discussed the possibility that the latter changes might be related to changes in the turgor pressure of the tissue: a salt solution would be expressed at a rate which varies according to the magnitude of the turgor pressure. This is interesting in connection with HAGAN's paper (1949). This author determined the negative exudation of wilted Helianthus plants, that is the rate at which water was sucked in through the stump. The negative exudation proved to be maximal during the night and minimal by day. In the latter case positive exudation might even occur. This could happen when the plants were in soil, and also if they were in a moist room, when no water could be taken up. The cycles disappeared if N_2 or CO_2 was present in the medium, but the negative exudation continued, although at a slower rate. HAGAN considers changes in the hydration of the protoplasm responsible. These experiments at any rate show that the root cells can express liquid. HAGAN did not investigate whether water or a salt solution was exuded.

BÜNNING (1942) demonstrated that various periodicity phenomena were accompanied by changes of turgor pressure. ENDERLE (1951) showed that the turgor pressure of carrot tissue could change periodically. According to BÜNNING this would be caused by fluctuations in the production of respirable substrate. These fluctuations would result from changes in the activity of the enzymes concerned. Changes in the intensity of the respiration would exercise influence on the enzyme activity and so on. BÜNNING (1951) found with several plants that the activity of the enzymes concerned with the synthesis of cellulose showed such fluctuations. He assumes that an endogenous rhythm is involved, which can be affected by illumination during a certain sensitive period.

SPEIDEL (1939) tried to explain the periodicity of exudation also by assuming changes in the production of respirable substrate, eventually caused by changes in the activity of enzymes. SKOOG, BROYER and GROSSENBACHER, however, found no relation between changes in exudation and respiration. SPEIDEL's hypothesis of the causes of exudation has not found much support.

If both secretion processes change periodically, the hypothesis of a fluctuating turgor pressure is insufficient. It might be that metabolic activity changes during the day, but there are few relevant data, beside those of BÜNNING and his collaborators. We may call attention to the investigation of VIRGIN (1951). VIRGIN found that the leaf cells of Helodea densa showed a maximal viscosity during the night, while

viscosity was minimal by day. This rhythm could be maintained over several days. Changes in the periods of illumination could influence the time at which maxima and minima occurred. VIRGIN demonstrated that the changes in viscosity ran parallel to those in permeability under certain circumstances. It seems possible to explain the changes of both water and salt transport as a result of changes in viscosity. However, there is nothing known as yet about the occurrence of such changes in the plasm of root cells.

V. SUMMARY

Starting from the supposition that the exudation is the result of a transport of salt to the vessels the salt secretion was investigated under various circumstances. For this purpose the rate of exudation was determined continuously, while the osmotic value of the sap was determined at certain instants. Also in some cases the water conductivity of the roots was determined. In most experiments tomato plants were used. Some experiments were repeated with Sanchezia nobilis.

The influence of the salt concentration of the medium on the salt secretion was studied first. Lowering the salt concentration of the outer solution caused a decrease of the rate of exudation, the course of the change depending on the extent to which the osmotic value of the medium had been simultaneously altered. From changes caused in this way it was concluded that the salts given off to the vessels must come partially from the root cells, while another part must have been transported directly to the vessels after having been taken up from the medium. If salts are present in the medium the latter process causes an increase in the exudation, but only if nitrates and chlorides are included. The importance of these anions for the exudation was demonstrated by determinations of the composition of the exudation sap. It appeared moreover that transport of phosphate to the vessels takes so much more time than that of nitrate and chloride that it suggests a different method of transport. The presence of nitrate or chloride in the outer solution always caused an increase in the exudation, no matter what cations were present.

The exflux of salts from the tissue — the tissue secretion — as well as the transfer to the vessels of salt taken up from the medium — the uptake secretion — was inhibited by inhibitors like potassium cyanide, dinitrophenol, sodium arsenate, sodium fluoride, iodo acetic acid and moreover phenylurethane. This indicates that both processes partially coincide. Their common part might be the transport of salts through the symplast. The water conductivity was also affected by these substances.

Sugar proved to promote the exudation only if salts were present in the medium.

The fact that the tissue secretion depended on the osmotic value of the medium led to the supposition, that salt and water are given off together from the tissue. An osmoregulation of the movement of

water must take place afterwards, the osmotic value of the sap being influenced by that of the outer solution; that is to say that water is sucked in by the solution given off from the tissue to the vessels. It was not possible to decide whether salts taken up from the medium are also transported with water. This process seemed independent of the osmotic value of the medium.

Rate of exudation, salt secretion and water conductivity of the roots were shown to suffer periodic fluctuations, a maximum occurring by day, a minimum during the night. A relation between the time at which maxima and minima occurred and certain conditions during the experiments or the period of cultivation could not be demonstrated.

The changes in the rate of exudation observed under certain conditions were in agreement with the supposition that the exudation consists of a transport of salt and a transport of water, the roots acting as an osmometer. A less satisfactory agreement between the osmotic value of the sap calculated according to this hypothesis and the value determined was sometimes found. In most cases too low a value was actually found, in some, however, the osmotic value of the exudation sap was higher than would be expected. The possible causes of this deviation have been discussed.

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