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THE UPTAKE OF CATIONS BY VALLISNERIA LEAVES

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CONTENTS

CHAPTER I

INTRODUCTION

1. Statement of the Problem

When a plant is transferred from water to a salt solution, there is a short period of rapid uptake of ions, which is followed by a slower uptake. It is generally accepted that the slow uptake depends on metabolism and that the ions are mainly accumulated in the vacuole, whereas the initial rapid uptake is mainly non-metabolic. Characteristics of uptake that depend on metabolism are that it is inhibited by low oxygen tensions, by low temperatures and by certain poisons. Opinions differ as to the question which role is played by metabolism in the initial rapid uptake. The notion of a Free Space, that is, a compartment of the cell which is freely accessible for ions by diffusion, implies that part of the initial uptake is non-metabolic. Because it was not certain that, in this space, ions are actually free Hope and Stevens (1952) introduced the term Apparent Free Space (A.F.S.). Briggs and Robertson (1957) divided the A.F.S. in a "Water Free Space" and a "Donnan FreeSp ace". Ions would move freely in the W.F.S., but in the Donnan Free Space they would be held by coulomb forces. Usually, the amount of cations taken up during the initial rapid diffusion exceeds the number of anions, due to an exchange of cations of the D.F.S. for cations from the outer solutions.

One of the many theories about the mechanism of ion absorption by living cells is the carrier theory. This theory starts from the hypothesis that the ions are bound to some movable compound of the cytoplasm, which can permeate through a membrane that is impermeable for free ions. In this way ions might be absorbed by the living cell.

Van den Honert (1933, 1936) belonged with Stiles (1924) and Lundegårdh (1932) to the first, who supported the theory, that uptake of ions starts with adsorption to a cytoplasmic compound. He showed that, in phosphate absorption by sugar cane, the absorption rate-concentration curves resemble Freundlich's adsorption isotherm. For this reason Van den Honert assumed that the first step in the uptake was adsorption of the phosphate ions. The second phase of uptake would be translocation of the adsorbed ions into the cell. This phase is temperature-sensitive, and, hence, Van den Honert concluded that it depends upon metabolism. He compared the uptake mechanism to a conveyer belt with a constant loading capacity and a rotation velocity regulated by metabolism.

Osterhout (1936), Hoagland and Broyer (1936) and Arisz (1944, 1945) also pointed to the significance of adsorption processes for the absorption of compounds by the cytoplasm Jacobson *et al.* (1950) and Overstreet *et al.* (1952) developed this hypothesis further by advancing the possibility that ions might react with metabolically formed cytoplasmic compounds under delivery of

hydrogen and hydroxyl ions. The complexes formed would be decomposed by a chemical change after passing the barrier.

EPSTEIN and HAGEN (1952), though not starting from a delivery of hydrogen and hydroxyl ions, based their experiments also on the hypothesis that the absorption process implies the formation and breakdown of a complex of an ion and a metabolically formed carrier. They found that quantitatively the absorption of alkali cations is in accordance with the view that a binding in the form of a labile complex occurs in the absorption process.

The relationship can be expressed by the equations

$$R + M \text{ outside} \underset{K_2}{\overset{K_1}{\rightleftarrows}} MR$$

$$MR \underset{K_4}{\overset{K_3}{\rightleftarrows}} R + M \text{ inside}$$

Moreover their experiments point to the existence of specific binding sites each preferring a special ion.

HELDER (1952) also showed that, with intact maize plants, the relationship between concentration and absorption of ions could be represented by an adsorption-isotherm (a Freundlich or a Langmuir curve). As it is known of many enzymatic processes that the relationship between reaction velocity and the concentration of the substrate can also be expressed by a Langmuir equation, he suggests that the first step of the absorption of phosphate is enzymatic.

It has been ascertained for several plant tissues, which are able to absorb ions, that they bind ions which can be removed again by exchange for other ions. This holds especially for the cations. (DEVEAUX, 1896; MAZIA, 1938; BROOKS, 1938, 1939; STEWARD and HARRYSON, 1939; BUTLER, 1953; EPSTEIN, 1954; BRIGGS, HOPE and PITMAN, 1958; DAINTY and HOPE, 1959). This process of reversible binding of ions is identified by many investigators with the binding to cytoplasmatic compounds according to the carrier hypothesis. Also when they are not inclined to identify the exchange-sites with carriers, these investigators consider the binding to the exchange-sites a necessary step in the absorption of ions. (JACOBSON, 1950; SUTCLIFFE, 1954; HIGINBOTHAM and HANSON, 1955; RUSSELL and AYLAND, 1955; LUNDEGÅRDH, 1958; GONZALES and JENNY, 1958).

Contrary to this view, is the opinion of EPSTEIN and LEGGETT (1954), LATIES (1959) and LAGERWERFF (1961), who attribute little or no value to the exchangeably bound ions for the absorption process.

The aim of the present research was to try, at least with *Vallisneria* leaves, to obtain decisive information about the significance of the exchangeably bound ion fraction for the absorption process.

2. LITERATURE

According to GONZALEZ and JENNY (1958) roots of Alfalfa seedlings absorb cations by surface migration. They demonstrated that Sr ions

which were bound by a cation exchange resin, could be absorbed by roots which were in contact with the resin.

It is true that these experiments proved that cation absorption by plant cells was possible by means of exchange, but it was not proved that under different conditions the exchange step is necessary for the absorption of ions. Moreover it was not demonstrated that the Sr ions, once absorbed, could not be exchanged for other cations. Absorption, in their experiments, may have consisted for one part of an irreversible and metabolically regulated uptake and for the other part of a reversible adsorption.

HIGINBOTHAM and HANSON (1955) studying the absorption of rubidium by discs of potato tissue found the same relationship between initial uptake (adsorption exchange) and the concentration of the external solution as between accumulation and that concentration. The curve representing this relationship resembled Freundlich's adsorption isotherm.

They considered this to be an indication that the adsorption exchange is the first, and hence a necessary step in the process of ion uptake. The accumulation would depend on the adsorption exchange fraction, and the latter would depend on the concentration of the outer solution. It will be clear that the fact that initial uptake and the accumulation process bear the same relationship with the concentration of the outer solution does not prove that the initial uptake is a necessary step in the uptake process. The similarity of this relationship in both processes may be a mere coincidence.

LUNDEGÅRDH (1958) studying the uptake of potassium and chloride by wheat roots also found a similar relation between initial absorption and concentration of the outer solution and between the accumulation rate and that concentration. He too concluded that the initial absorption limits the accumulation rate. (According to Lundegårdh adsorption exchange is the principal mechanism of the initial absorption). In Lundegårdh's experiments as in earlier investigations, the accumulation started slowly and it only reached its maximal rate after 30 minutes. However, the initial uptake reached 40–80 % of its maximal value after 5 minutes and its maximum within 15 minutes. This makes it improbable that the initial uptake is a necessary phase of the accumulation process. Contrary to Lundegårdh's results EPSTEIN (1954) and WINTER (unpublished) found that the accumulation rate attains a constant value within 5 minutes from the start of the experiment.

Scott RUSSELL and AYLAND (1955) assume that the rate of the initial rapid uptake is determined by exchange reaction's since the uptake of rubidium was reduced in the presence of other ions.

They conclude that, if adsorption to carriers exists in the sense of JACOBSON and OVERSTREET (1947), it will take place after the initial entry of the cations by exchange. To this the objection may be made that the fact that exchange reactions are rate-determining in the initial rapid uptake does not prove that the same reactions determine the rate of the subsequent slow accumulation process.

Many soil scientists assume that the uptake of cations by roots and the selectivity of this uptake are determined by the exchange capacity of the root system. Often these conclusions are invalidated by the fact that exchangeable cations and cations that are irreversibly accumulated have not been determined separately in their experiments (e.g. ELGABALY and WIKLANDER, 1949).

3. MATERIAL AND METHODS

The experimental plant, *Vallisneria spiralis*, was cultivated in concrete basins of 110 × 110 × 50 cm which stood in the basement of the laboratory. The roots grew in a 10 cm layer of clay on top of which a thin layer of sand had been placed to prevent the water from being polluted by clay particles. The basins were to the edge filled with demineralized water. The plants were illuminated by means of a Philips 450 Watts mercury vapourlamp for 15 hours daily. The temperature of the water oscillated between 22° C and 24° C.

For uptake experiments the leaves were cut into 2,5 cm segments. The edges were trimmed off leaving a strip of 2,5 × 4 mm. The strips were randomized and divided into sets of eight. Each set was mounted in a small perspex frame (Fig. 1).

The capacity for ion uptake of leaf-strips in light increases during the first hours after cutting, due to recovery from wounding. (ARISZ, 1948, 1957; SOL, 1958). For that reason the strips were pretreated in demineralized water for 24 hours. During pretreatment the strips were illuminated by means of an incandescent bulb of 100 Watts at a distance of 50 cm. The water was constantly aerated with air that had been freed from carbon dioxide. The temperature was kept constant at 25° C.

For the determination of ion-uptake two sets were placed in a perspex vessel with parallel walls (Fig. 1). The vessel contained

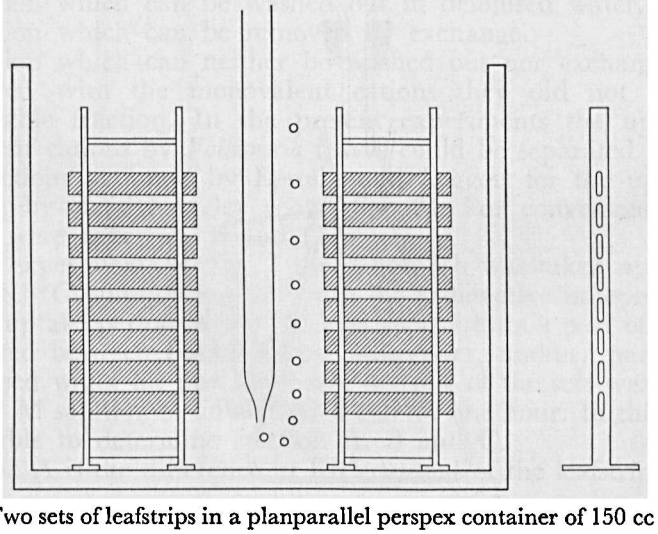

Fig. 1. Two sets of leafstrips in a planparallel perspex container of 150 cc capacity.

125 ml solution. Conditions during uptake were the same as during the pretreatment.

Uptake was determined by means of tracer methods. Rubidium, calcium, chloride and sulphate were labelled with Rb–86, Ca–45, Cl–36 and S–35.

The leaf strips were digested in 2 ml of concentrated nitric acid on a water-bath at 96° C for 2–3 hours. If chloride had to be determined, silver nitrate was added to the nitric acid to prevent the loss of hydrochloric acid during digestion. When the digestion was completed the liquid was made up to a volume of 25 ml with water. Rubidium and chloride were determined in 10 ml of the extract by means of a dipping Geiger–Müller tube. A control solution of known specific activity was counted in the same way. Uptake was computed from the difference of the two countings.

For the determination of sulphate uptake the leaf-strips were not digested but boiled in inactive K_2SO_4 (250 μ mol.). The active sulphate ions in the leaves are exchanged for inactive ions.

It is possible to obtain a 10 percent higher yield of active sulphate from the leaves by ashing with Na_2CO_3 and Na_2O_2 (PAECH, 1955).

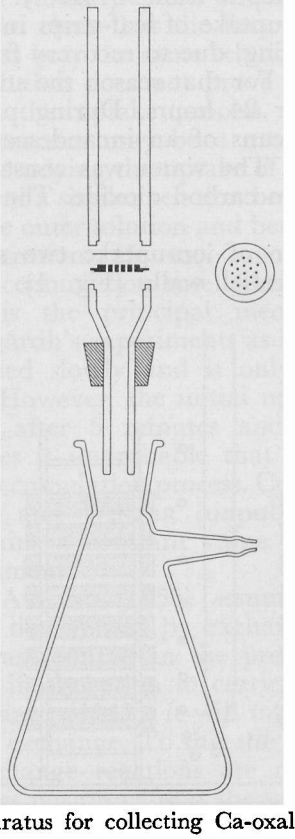

Fig. 2. Apparatus for collecting Ca-oxalate precipitates.

Since differences in uptake only were important the less cumbersome extraction by exchange has been preferred. After extraction the sulphate solution was acidified and the sulphate was precipitated as $BaSO_4$. The precipitate was collected on a filter (Schleicher and Schüll, Blue band, nr. 589/3). The filter was ashed in an oven at 620° C. The barium sulphate was suspended in 96 % ethyl alcohol and transferred to an evaporation dish. After evaporation of the alcohol under an infra-red lamp a layer was obtained which contained 12,86 mg/cm². Its activity was measured by means of a window-less flow counter. Uptake was measured from the difference in counts per minute between the sample and a sample of known specific activity that had been prepared in a similar way.

Calcium was precipitated by adding potassium oxalate after addition of 6,54 mg of calcium chloride to the digest and neutralisation with 20–25 % ammonia, (ILJIN, 1939). The added calcium served as a carrier for the radio-active ions. The precipitate was collected on a Schleicher and Schüll Blueband filter, nr. 589/3 (Fig. 2). After drying by means of an infra-red lamp an even layer remained which contained 2,43 mg/cm². The activity was determined by means of an end-window Geiger–Müller tube. Uptake was computed from the difference of this counting and that of a similarly treated precipitate of known specific activity.

CHAPTER II

PRELIMINARY EXPERIMENTS

1. UPTAKE OF RUBIDIUM

As was mentioned already in the introduction, EPSTEIN and LEGGETT (1954) succeeded in separating the Sr absorbed by excised barley roots into three fractions namely:

a fraction which can be washed out in deionised water.
a fraction which can be removed by exchange.
a fraction which can neither be washed out nor exchanged.

However, with the monovalent cations they did not find an exchangeable fraction. In the present experiments the uptake of monovalent cations by *Vallisneria* leaves could be separated into the same fractions as found by Epstein and Leggett for the uptake of strontium by excised barley roots (Fig. 3). For convenience these fractions were called A, B and C.

In the experiments of Fig. 3 the cation Rb was taken up from a .008 M Rb*Cl solution labelled with the radioactive isotope Rb–86. After an uptake period of 60, 120 and 240 minutes a part of the sets was blotted between double-folded filterpaper, another part rinsed in deionised water for one hour and the rest of the sets was bathed in a .008 M solution of unlabelled RbCl for one hour. In this way it was possible to determine fraction A, B and C.

Fraction A is the difference in Rb* content of the leafstrips which

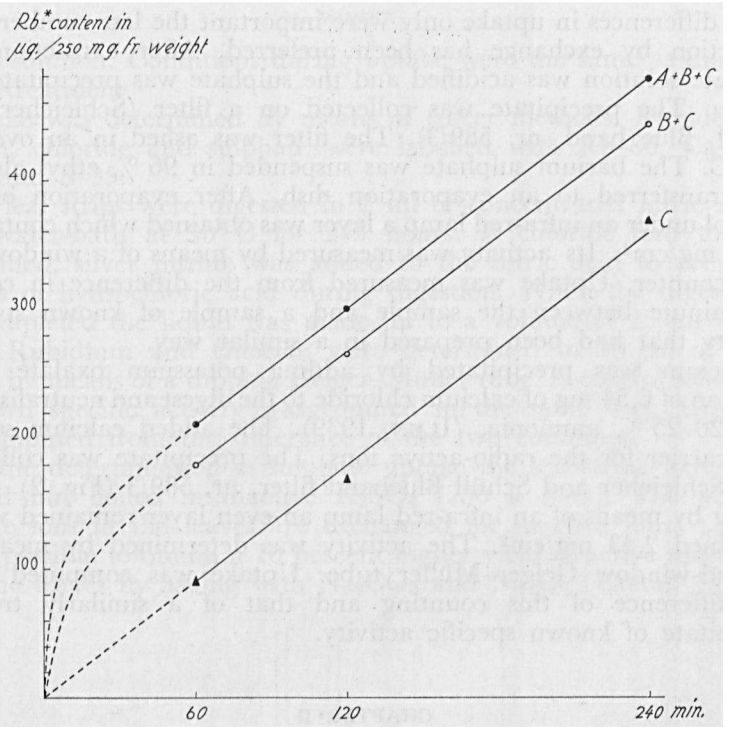

Fig. 3. Rubidium content of leafstrips after absorption from a .008 M Rb*Cl solution for various periods. Treatment of the leaves after removal from the Rb*Cl solution: Blotted (A + B + C); in water for one hour (B + C); in unlabelled RbCl for one hour (C). Each point represents the average value of two replications.

were blotted between double-folded filterpaper and the leafstrips which were rinsed in deionised water for one hour.

Fraction B is the difference in Rb* content of the leafstrips which were rinsed for one hour in deionised water and the leafstrips which were bathed for one hour in a solution of unlabelled RbCl. It contains the exchangeable ions.

Finally fraction C is the quantity of Rb* which remains in the leafstrips after bathing them in a solution of unlabelled RbCl. Fig. 3 shows that fraction C is linearly proportional to time but that on the other hand the fraction A as well as fraction B rapidly reach their maximum value. This value is reached within 60 minutes. The points in Fig. 3 are the averages of two sets. Within a single experiment replicates did not differ more than 5–10 percent.

2. Uptake of Chloride and Sulphate

Vallisneria leaves were bathed in a KCl* solution .008 M labelled with Cl-36. After a sixty minutes uptake period the fraction A, B and C were determined as has been described for the Rb ion.

Fig. 4 shows the Cl uptake. This figure gives the results of three different experiments a, b and c. The length of the columns gives the content of labelled chloride of the leaves at the end of the experiments. The shaded column gives the sum of the fraction A, B and C. The white column the sum of the fractions B and C and the black column gives the fraction C. In Fig. 4a and b there is a

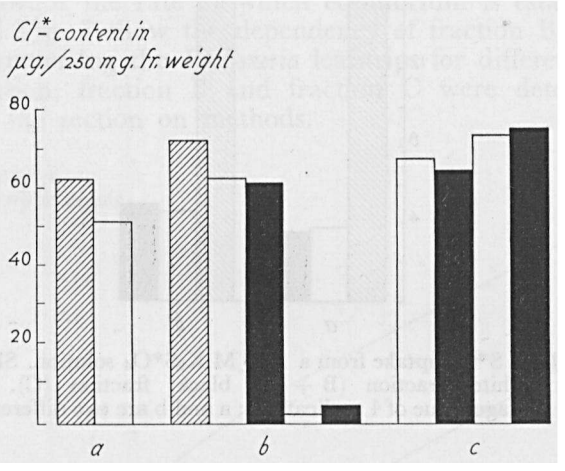

Fig. 4. One hour Cl* uptake from a .008 M KCl* solution. Shaded: fraction (A + B + C); white: fraction (B + C); black: fraction C. Each column represents the average value of two replications; a, b and c are 3 different experiments.

significant difference between the shaded column and the white column indicating that for chloride a fraction A exists. On the contrary there is no significant difference in length of the white and the black column in Fig. 4b and c. Thus if for chloride a fraction B exists it is very small as compared to the fraction B for cations. Because fraction B is determined as a difference, its absolute error is smaller when the values, from which it is determined, are low than when these values are high. For this reason fraction B was determined at 5° C. The two last columns of Fig. 4b give the result. Fraction B was even negative, but the value is not significant. The results with sulphate are shown in Fig. 5.

Owing to the fact that the specific activity of the sulphate solutions was higher than that of the chloride solutions, differences in uptake of sulphate could be determined with a higher accuracy than the differences in chloride absorption. With chloride a fraction B could only be determined if it was of the order of a few µg/250 mg fresh weight, with sulphate the smallest quantity was as low as 0,1 µg/250mg fresh weight. Fig. 5 shows that for sulphate ions a fraction B could not be detected. Sulphate was taken up from a .008 M solution. Each column in Fig. 5 is an average of 4 sets, representing one gram of leaves fresh weight.

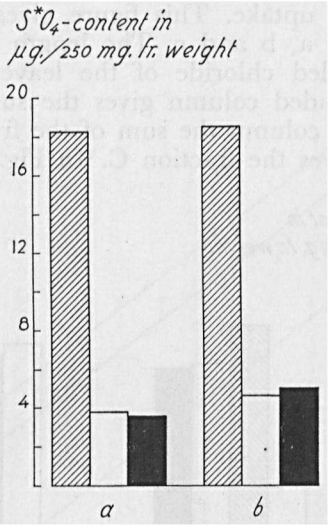

Fig. 5. One hour S^*O_4 uptake from a .008 M $K_2S^*O_4$ solution. Shaded: fraction (A + B + C); white: fraction (B + C); black: fraction (C). Each column represents the average value of 4 replications; a and b are two different experiments.

CHAPTER III

THE DEPENDENCY OF THE Rb FRACTION B ON METABOLIC ACTIVITY

In the literature on ion-uptake metabolic absorption and exchange-absorption are generally distinguished.

EPSTEIN and LEGGETT (1954) have summarized the properties of these two modes of uptake as follows:

	Exchange adsorption	*Active transport*
1	Non linear with time; equilibrium approached in 30 min.	Linear with time; no equilibrium reached in the experiment.
2	Ions readily exchangeable.	Ions essentially non exchangeable.
3	Not selective with respect to various ions.	Selective with respect to various ions and groups of ions.
4	Requires no energy expenditure on the part of the tissue.	Requires energy expenditure.

In the previous chapter it was shown that the fraction B is readily exchangeable as opposed to the fraction C which is given off neither to water nor to a salt solution. Loss of measurable amounts from the fraction C to the surrounding solution only happens when the condition of the *Vallisneria* leaves declines. A very sensitive indication of less favourable condition is the infiltration of the intercellular spaces with liquid. When the *Vallisneria* leaves are in a good condition the septa in these spaces are permeable to gases but not to water (SOLEREDER, 1913).

The experiments of this chapter were carried out to check if our fraction B conforms to Epstein and Leggett's requirements for exchangeadsorption.

1. The rate of establishment of the equilibrium

A second point of difference between exchange adsorption and active transport is the rate at which equilibrium is established.

Fig. 6 and Fig. 7 show the dependency of fraction B and C on time. After immersing the *Vallisneria* leafstrips for different times in a RbCl solution, fraction B and fraction C were determined as described in the section on methods.

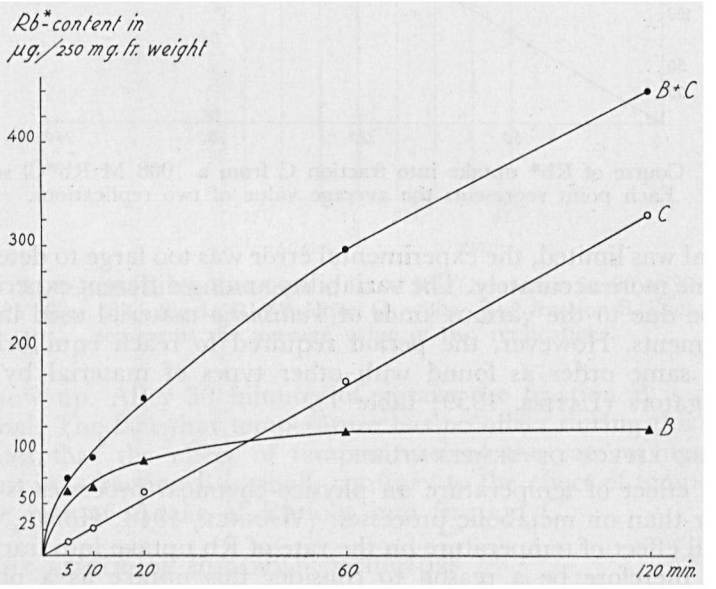

Fig. 6. Course of Rb* uptake from a .008 M Rb*Cl solution. B and C refer to the fraction B and fraction C. Each point represents the average value of two replications.

The uptake of Rb-ions into the fraction C appeared to be constant during the first hour of the experiment (Fig. 6). Under favourable circumstances such constant rate could be maintained during several hours (Fig. 7). We can also see that in *Vallisneria* leaves there exists no significant lag period in the uptake of Rb-ions into fraction C as was found by Lundegårdh (1958) with wheat roots.

The uptake of Rb-ions into fraction B is not linear with time. The rate of uptake during the first 5 minutes is much greater than the rate of uptake into fraction C in the same period, but it decreases very quickly. We can safely assume this rate to be zero after a sixty minute period, and the equilibrium to be established. In the greater part of the experiments equilibrium was found to be established within 30 or 60 minutes. As the amount of material I had at my

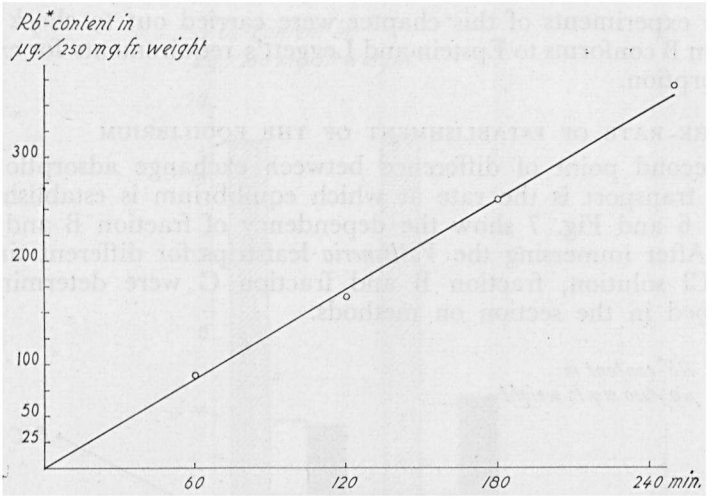

Fig. 7. Course of Rb* uptake into fraction C from a .008 M Rb*Cl solution. Each point represents the average value of two replications.

disposal was limited, the experimental error was too large to determine this time more accurately. The variability among different experiments may be due to the various kinds of *Vallisneria* material used in these experiments. However, the period required to reach equilibrium is of the same order as found with other types of material by other investigators (LATIES, 1959; table 1).

2. THE EFFECT OF TEMPERATURE

The effect of temperature on physico-chemical processes is much smaller than on metabolic processes. (WANNER, 1948; HÖBER, 1945). A small effect of temperature on the rate of Rb uptake into fraction B would therefore be a reason to consider this uptake as a physico-chemical process.

Fig. 8 shows the effect of temperature. The uptake proceeded in the dark at 25° C and 5° C for 60 minutes and 30 minutes. In other experiments it was found that the rate at which Rb ions are accumulated in fraction C is much smaller in the dark than in the light. On the other hand fraction B is not influenced by light or dark. If uptake is studied in the light, fraction C is large and an error in its determination will effect the difference between (B + C) and C (i.e. fraction B) much more than when fraction C is small. Therefore, the advantage of studying the uptake in the dark is that the size of the fraction B can be determined more precisely, for the fraction B is determined by measuring the difference between the fractions (B + C) and C. After 60 minutes or after 30 minutes uptake hardly a significant effect of temperature on the rate of uptake of Rb-ions into fraction B could be shown. A 60 minutes uptake period is more than sufficient for the fraction B to reach its maximum value at a temperature of 25° C and, therefore, an effect of temperature might

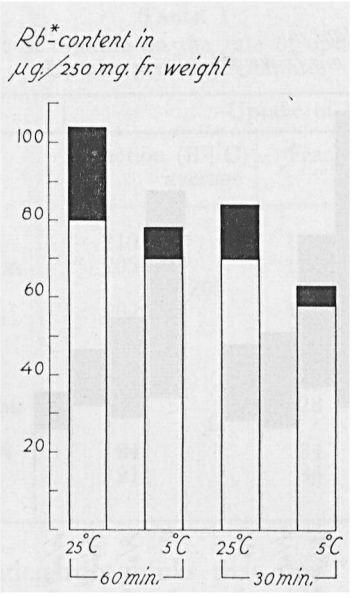

Fig. 8. Rb* uptake in the dark from a .01 M Rb*Cl solution for 60 or 30 minutes and at 25° C or 5° C. Black: Rb* fraction C; white: Rb* fraction B. Each column represents the average value of two replications.

not show up. After 30 minutes of uptake the fraction B is not yet maximal. The fact that temperature has no effect during this period indicates that the effect of temperature on the rate of uptake of Rb-ions into fraction B is small, contrary to the effect of temperature on the rate of uptake of Rb-ions into fraction C.

3. The effect of metabolic inhibitors

Inhibitors which strongly influence metabolic processes are also applied to distinguish between both uptake fractions. If the uptake into fraction B is an exchange-adsorption and the uptake into fraction C depends on metabolism, the rate of uptake into C will be decreased by metabolic inhibitors, whereas the rate of uptake into fraction B will not be influenced by these compounds.

In the first place the effect of HCN was studied, Arisz (1953, 1956) and Van Lookeren Campagne (1957) examined in detail the effect of HCN on the chloride uptake by *Vallisneria* leaves and their experiments showed among other things that the chloride uptake is strongly inhibited by HCN. It was to be expected that, directly or indirectly, also the cation uptake would be affected by HCN, Lundegårdh and Burström (1935). Fig. 9 shows three experiments in which the effect of different concentrations of HCN on the Rb uptake was investigated. The HCN stock-solution was made from a KCN solution by adjusting the acidity of the solution to pH 7 with the aid of HCl. The leaves in these experiments were not pretreated with HCN as it works almost instantaneously. This followed from other experiments

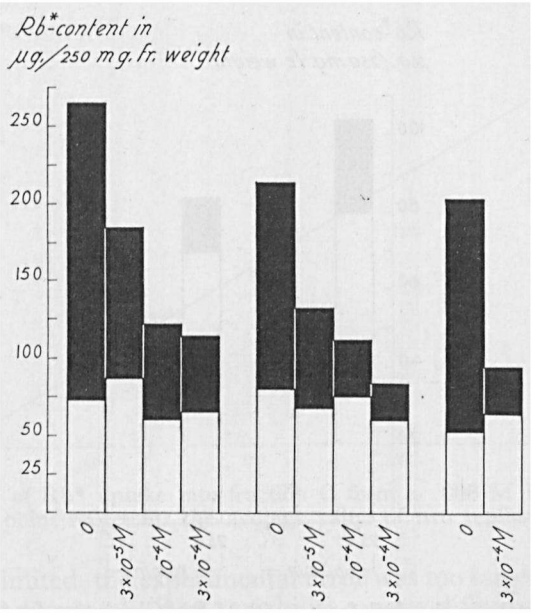

Fig. 9. Three experiments on Rb* uptake from a .008 M Rb*Cl solution for 60 minutes in the presence of 0, 3×10^{-5}, 10^{-4} and 3×10^{-4} M KCN. Black: Rb* fraction C; white: Rb* fraction B. Each column is the average value of two replications with the exception of the third experiment where each column represents the average value of 4 replications. KCl was added to the control to eliminate the effect of the K ions of the KCN solution on the Rb* fraction B.

in which the leaves were pretreated with HCN. Thus, it was sufficient to add the HCN only during the uptake period. By the HCN treatment the rate of uptake of the Rb-ions into fraction C is strongly inhibited, whereas fraction B is hardly influenced. The differences in size are not significant. Table 1 gives an impression of the variability of the determinations. The last two columns of Fig. 9 are derived from these values.

SUTCLIFFE (1954) and LAGERWERFF (1956) could not find any effect of respiration-inhibitors on adsorption-exchange. On the other hand there are given in literature a few examples in which HCN affects the Apparent Free Space. (BERGQUIST, 1958; LUNDEGÅRDH, 1958). Fraction A and fraction B as defined in the foregoing are identical with the Apparent Free Space of HOPE and STEVENS (1952).

BERGQUIST (1958) showed that in the Brown-algae *Hormosira banksii* HCN increases the fraction A as well as the fraction B instead of decreasing it.

LUNDEGÅRDH (1958) however found that HCN inhibits the "Initial absorption" of Cl and K by wheat roots. He concludes from this that the "Initial absorption" is not completely non-metabolic. The "Initial absorption" is the first rapid uptake of ions by plant material when this is placed in a salt solution, so it can include, fraction A as well

TABLE 1

The effect of cyanide on the rate of uptake of Rb*.
Uptake period 60 minutes

External solution	Uptake of Rb in μg.					
	Fraction (B+C)	average	Fraction C	average	Fraction B	average
.008 M Rb*Cl solution	210		158		52	
	205		153		52	
		205		153		52
+ 3 × 10^{-4} M KCl.	207		156		51	
	198		146		52	
.008 M Rb*Cl solution	106		29		77	
	91		28		63	
		94		31		63
+ 3 × 10^{-4} M KCN	84		34		55	
	91		34		57	

as fraction B. Lundegårdh thinks that the "Initial absorption" is mainly adsorption-exchange, that would correspond with our fraction B.

The possibility exists, of course, that it is not allowed to compare a fraction B of one object with the fraction B of another, as the only common feature might be the exchangeability. Lundegårdh finds an adsorption-exchange of anions as well as for cations. Generally it is found however (KYLIN and HYLMÖ, 1957; LEGGETT and EPSTEIN, 1956), that the adsorption-exchange for anions is so small that it is hardly detectable. The discrepancy between the findings of Lundegårdh and other investigators can not be easily explained. However, whether the "Initial absorption" of Lundegårdh exists of a fraction A, a fraction B or a combination of both, the fact remains that HCN in his experiments, as opposed to mine with *Vallisneria* leaves, strongly affects the first rapid uptake of ions.

Just as HCN, the metabolic inhibitor Uranylnitrate strongly affected the rate of uptake of Rb-ions into fraction C and not the rate of uptake into fraction B (Fig. 10). Uranylnitrate was used because it was found by ARISZ (1958) that the chloride absorption was inhibited by Uranylnitrate. Uranylnitrate was added during the pretreatment as well as during the uptake period. In preliminary experiments it was found that it does not work immediately but only when it had been into contact with the leafstrips for several hours. The differences which are found in the reduction of the fraction B after treatment with the inhibitor are not significant.

Finally an attempt was made to influence the rate of absorption into fraction B with monoiodoacetamide, the inhibiting action of which on metabolic processes is a.o. based on combination with reactive SH groups. As with the other inhibitors the rate of uptake of Rb-ions into fraction C was nearly completely inhibited, but there

was no significant effect on the rate of uptake into fraction B (Fig. 11).

We may conclude from this chapter that whereas the formation of the fraction B depends upon physico-chemical features of the plant cell, the formation of the fraction C is dependent on its biochemical activity.

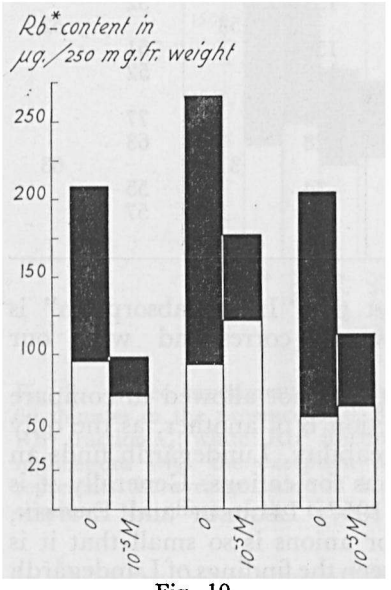

Fig. 10

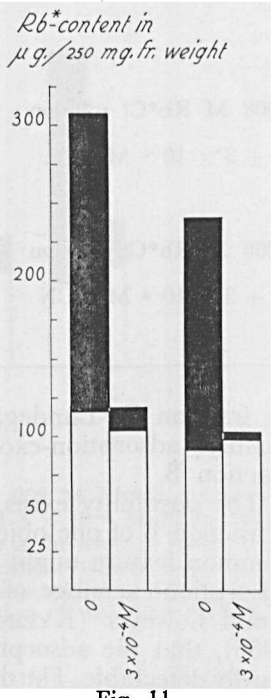

Fig. 11

Fig. 10. Three experiments on the effect of uranylnitrate on Rb* uptake from a .008 M Rb*Cl solution for 60 minutes. Black: Rb* fraction C; white: Rb* fraction B. Each column gives the average value of two replications.

Fig. 11. Rb* uptake from a .008 M Rb*Cl solution for 60 minutes after a two hours pretreatment in the dark in water or in a solution of 3×10^{-4} M monoiodoacetamide. Black: Rb* fraction C; white: Rb* fraction B. Each column represents the average value of two replications.

CHAPTER IV

THE RELATIONSHIP BETWEEN FRACTION B AND THE CONCENTRATION OF THE SURROUNDING SOLUTION

When leaves of *Vallisneria* are bathed in Rb-solutions the relationship between the fraction B and the external concentration proves to be nearly linear (Fig. 12).

When a fraction B of labelled rubidium ions has been established it is possible to remove the labelled ions by exchange for unlabelled rubidium. Fig. 13 shows that this exchange takes place in outer

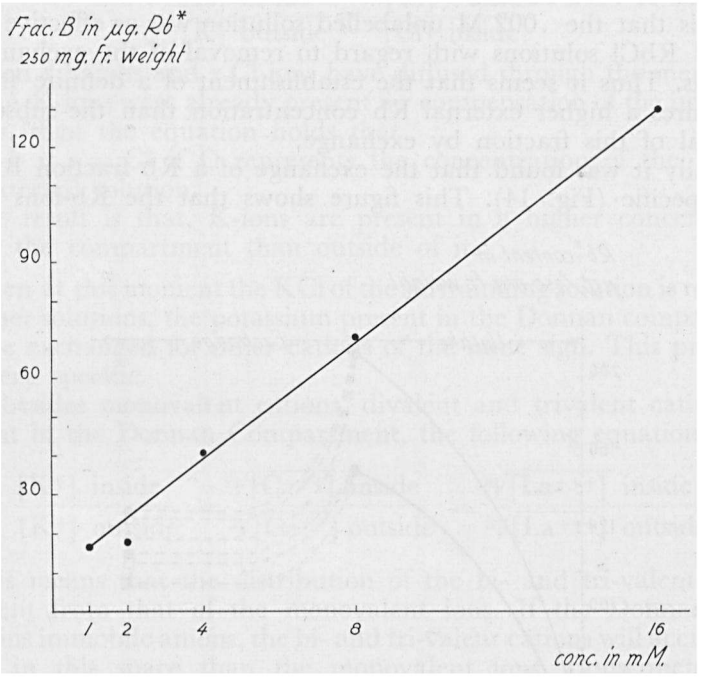

Fig. 12. The effect of the concentration of Rb*Cl on the size of the Rb* fraction B. Uptake for 60 minutes. Each point represents the average value of two replications.

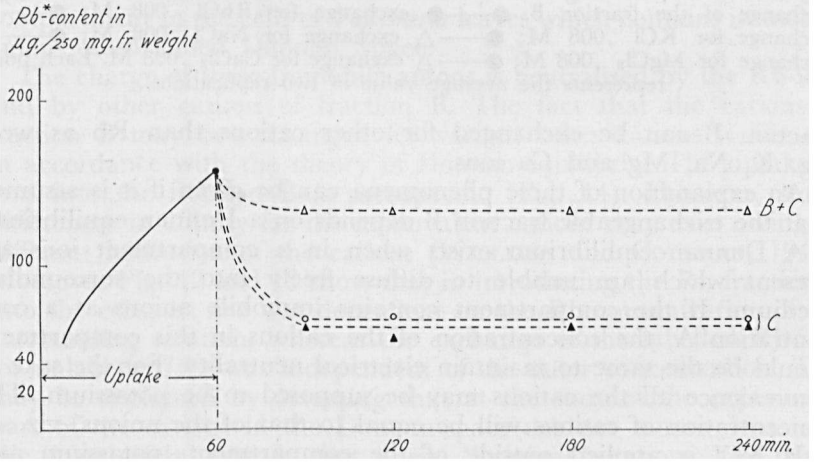

Fig. 13. Effect of the concentration of the unlabelled RbCl solution on the exchange of Rb* ions from fraction B. B and C refer to the fraction B and fraction C. ○——○ exchange in a .002 M RbCl solution; ▲——▲ exchange in a .010 M RbCl solution. Each point represents the average value of two replications.

solutions of .002 M and .010 M at the same rate. The most striking result is that the .002 M unlabelled sollution was as effective as the .01 M RbCl solutions with regard to removal of the exchangeable Rb-ions. Thus it seems that the establishment of a definite fraction B requires a higher external Rb concentration than the subsequent removal of this fraction by exchange.

Finally it was found that the exchange of a Rb fraction B is not very specific (Fig. 14). This figure shows that the Rb-ions of the

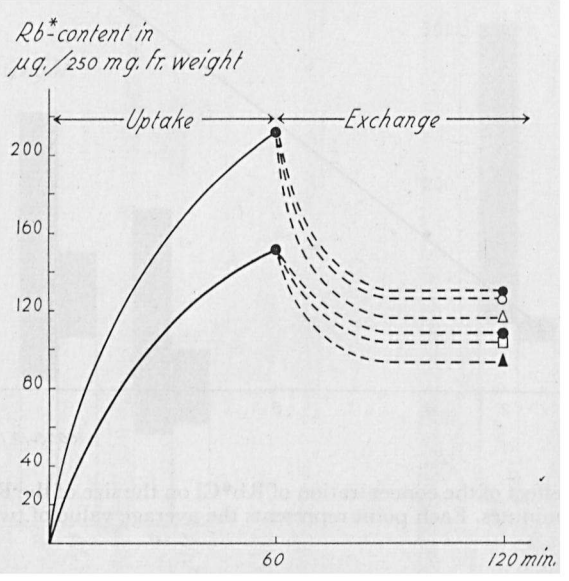

Fig. 14. Rb* uptake from a .008 M Rb*Cl solution for 60 minutes and subsequent exchange of the fraction B. ●——● exchange for RbCl .008 M; ●——□ exchange for KCl .008 M; ●——△ exchange for NaCl .008 M; ●——○ exchange for $MgCl_2$ .008 M; ●——△ exchange for $CaCl_2$ .008 M. Each point represents the average value of two replications.

fraction B can be exchanged for other cations than Rb as well, e.g. K, Na, Mg and Ca ions.

An explanation of these phenomena can be given if it is assumed that the exchangeable fraction B depends on a Donnan equilibrium.

A Donnan equilibrium exists when in a compartment ions are present which are unable to diffuse freely into the surrounding medium. If the compartment contains immobile anions at a concentration A, the concentration of the cations in this compartment should be the same to maintain electrical neutrality. For the sake of convenience all the cations may be supposed to be potassium. The concentration of cations will be equal to that of the anions, viz. A.

If KCl is applied outside of the compartment, potassium and chloride ions will diffuse into the space until the numbers of free ions which pass the boundary from left to right and vice versa are equal. These numbers depend on the products of concentrations of the ions. Thus:

$$\frac{[K] \text{ inside}}{[K] \text{ outside}} = \frac{[Cl] \text{ outside}}{[Cl] \text{ inside}}$$

When x K-ions and x Cl-ions have diffused through the membrane while A K-ions were already present for compensation of the immobile anions, then the equation holds that

$(A + x)\, x = C^2$ if C represents the concentration of the KCl in the external solution.

The result is that, K-ions are present in a higher concentration inside the compartment than outside of it.

When at this moment the KCl of the surrounding solution is replaced by other solutions, the potassium present in the Donnan compartment will be exchanged for other cations of the same sign. This process is not very specific.

If, besides monovalent cations, divalent and trivalent cations are present in the Donnan Compartment, the following equation holds:

$$\frac{[K^+] \text{ inside}}{[K^+] \text{ outside}} = \frac{\sqrt[2]{[Ca^{++}] \text{ inside}}}{\sqrt[2]{[Ca^{++}] \text{ outside}}} = \frac{\sqrt[3]{[La^{+++}] \text{ inside}}}{\sqrt[3]{[La^{+++}] \text{ outside}}}$$

This means that the distribution of the bi- and tri-valent ions is different from that of the monovalent ions. If the Donnan space contains immobile anions, the bi- and tri-valent cations will accumulate more in this space than the monovalent ions. Consequently the exchange of these ions for monovalent ions is more difficult than the reverse.

The ions in the Rb fraction B in *Vallisneria* leaves can be exchanged for other Rb-ions (Fig. 13). This is in agreement with the theory of Donnan equilibrium. If the theory is right there must be a compartment in the cells of *Vallisneria* leaves which contains immobile anions, a so called Donnan Space.

The charge of these immobile anions is neutralised by the Rb-ions and by other cations of fraction B. The fact that the cations of fraction B may be exchanged for various other cations is likewise in accordance with the theory of Donnan-equilibria. The uptake of ions into the fraction B is non-specific (Fig. 14). However, small differences in affinity for the monovalent cations may exist owing to different hydration of the cations (WILLIAMS and COLEMAN, 1949). Moreover Fig. 12 and 13 show that in introducing the Rb-fraction B into the cells, its size was dependent on the Rb concentration of the surrounding solution, whereas the exchange of the labelled Rb-ions for unlabelled Rb was independent of the outer concentration. This may be explained by assuming that at the start of the experiment part of the cations in the Donnan-space are not monovalent ions. We may safely assume that these "original" cations are partly calcium ions and, for a smaller part, magnesium-ions; as according to the Gibbs-Donnan distribution the Donnan-space has a higher affinity for calcium-ions than for the rubidium ions. Though the concentration of Ca outside of the Donnan-space will be extremely

small its concentration in the Donnan-space will remain relatively high as long as the ratio $\frac{[Rb]\text{-outside}}{[Rb]\text{-inside}}$ has a low value. This accounts for the strong dependence of the establishment of a fraction B on the Rb concentration of the outer solution.

If the Donnan-space contains Rb*-ions only, and these are exchanged for inactive rubidium ions (Rb°) equilibrium will be attained when

$$\frac{Rb^*}{Rb^\circ} \text{ inside} = \frac{Rb^*}{Rb^\circ} \text{ outside.}$$

Owing to the much larger volume of the outer solution as compared with that of the Donnan-space, the amount of unlabelled Rb will be much larger than the amount of labelled Rb even at low external concentration. Consequently virtually all of the Rb* of the Donnan-space will be exchanged for unlabelled rubidium of the medium. Only very low external Rb concentrations will be unable to remove the fraction B completely.

In order to test this theoretical explanation experiments were carried out in which the effect of a previous removal of the bivalent ions from the Donnan space was studied (Fig. 15). The open circles give the size of the Rb fraction B in leaves which had been treated with a .016 M solution of K_2SO_4 prior to the immersion in the solution of RbCl in order to remove most of the bivalent cations.

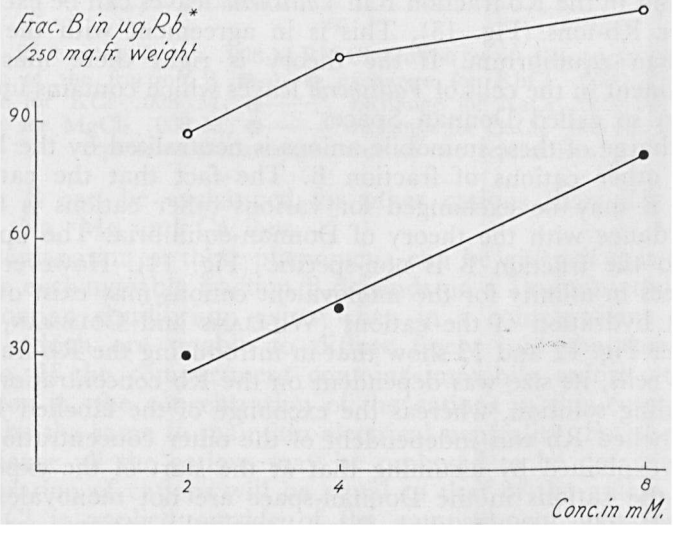

Fig. 15. The effect of a K_2SO_4 pretreatment on the size of fraction B. ●——● Rb* fraction B after uptake from Rb* solutions of different concentrations for 60 minutes. ○——○ Rb* fraction B after uptake from the same solutions by leaves that had been pretreated in a .016 M K_2SO_4 solution. Each point represents the average value of two replications.

The black dots give the size of the Rb fraction B of the control sets which had been bathed for a similar period in deionised water. Both sets were then allowed to absorb Rb from Rb*Cl solutions of various concentration for 60 minutes. The fraction B was determined in the usual way by exchange for unlabelled Rb. The fraction B of the control sets is almost proportional to the concentration of the labelled Rubidium solution.

The fraction B in the pretreated leaves is much higher than that of the leaves which had not been treated with K_2SO_4. Even in the lowest concentration of RbCl a large fraction B has been formed. Though in the higher concentrations of RbCl the fraction B is somewhat larger than in the lower concentrations, the linear proportionality has disappeared.

The small increase which was found may be ascribed to two causes. Possibly by the treatment of K_2SO_4, the bivalent ions originally present in the material, were not completely removed. Another possibility has been mentioned by BRIGGS, HOPE and PITMAN (1958). These authors assumed that the pH of the protoplasm increases with the concentration of the outer solution. This may cause an increase of the dissociation of the immobile anions in the Donnan-space, and, thus, an increase in number of the compensating counter ions, i.e. of the fraction B.

CHAPTER V

THE TRANSFER OF THE Rb FRACTION B TO THE FRACTION C AND ITS NATURE

1. THE TRANSFER OF THE Rb FRACTION B TO THE FRACTION C

In the previous chapters it was demonstrated that *Vallisneria* leaves possess an exchangeable cation fraction, the fraction B, just like numerous other plant tissues, e.g. roots, storage tissue, or algae. At the same time we tried to obtain an insight into the nature of this exchangeable cation fraction by studying the effect of some factors on this fraction. The second aim of this research was to study the relationship between the fraction B and the uptake of ions into the fraction C, in other words whether the fraction B is a link in the uptake of cations from the external solution into the fraction C.

First it was investigated if Rb-ions were transferred from fraction B to fraction C by a method after EPSTEIN and LEGGETT (1954).

Vallisneria leafstrips were placed in a RbCl solution .008 M and after an uptake period of 60 minutes in some sets the fraction B and the fraction C were determined. The other sets were transferred to deionised water after rinsing off the adhering salt solution. At different intervals, ranging from 15 min. to 120 min., some of the sets were taken out of the water and used for the determination of the fraction C. The result is shown in Fig. 16. The Rb fraction C increases during the time that the leafstrips are in the deionised water.

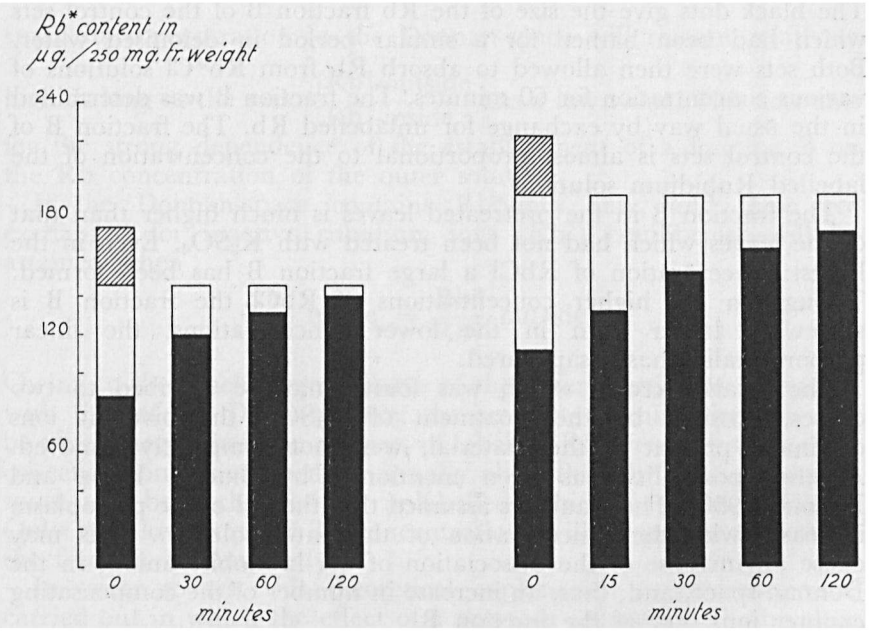

Fig. 16. Transfer of Rb* ions from the fraction B to fraction C in leaves kept in deionised water. Black: fraction C; white: fraction B; shaded: fraction A. Each point represents the average value of two replications.

Since the external solution has been removed this increase of the fraction C must be due to uptake of Rb-ions from fraction A, fraction B, or from both fractions.

However, the increase of the fraction C is such that it exceeds strongly the fraction A. Therefore, the increase cannot be ascribed to uptake of Rb-ions from the fraction A only. Before the leafstrips are transferred from the salt solution to deionised water, the greater part or perhaps the whole fraction A is washed out by dipping the leaves successively into four beakers with deionised water, to remove the adhering salt solution. This treatment takes 30 seconds. Then the leaves remain in deionised water for the time indicated in Fig. 16. It is assumed that the greater part of fraction A is removed by dipping the leaves in water during the first 30 seconds (LUNDEGÅRDH, 1949; HOPE, 1953). What remains of fraction A diffuses into the surrounding water. These ions might be taken up into the fraction C. The volume of water in which the leafstrips remained was 1500 cc. The total fresh weight of the strips was at most 4 gram, containing a total fraction A of about 500 μ grams. This would give a concentration in the medium of 333 μ grams per 1000 cc i.e. 4 micromols of Rb-ions. Uptake from such a low concentration would be practically negligeable in the time of the experiment. Therefore the increase of the fraction C must be due to a transfer of Rb-ions from the fraction B to fraction C. This phenomenon is not restricted to *Vallisneria* leaves.

EPSTEIN and LEGGETT (1954) already showed that an exchangeable Sr fraction of excised barley roots changed into an irreversible bound fraction. The rate of this change, however, was very slow. Also BROUWER (1959) showed that the exchangeable Rb fraction in excised pea roots could change into an unexchangeable fraction. Finally G. DE LEEUW 1957 (Unpublished results, Botanical Laboratory Groningen) found that the exchangeable Rb fraction in Beet-root disks (SUTCLIFFE, 1957) is transferred to fraction C.

2. THE EFFECT OF MONOIODOACETAMIDE

In chapter III we saw that monoiodoacetamide strongly inhibits the rate of uptake of the Rb-ions from the external solution into the fraction C. We investigated now whether or not monoiodoacetamide could inhibit the transfer of Rb-ions from the fraction B to fraction C. The advantage of monoiodoacetamide is that it has no cations which could exchange for the Rb-ions of the fraction B.

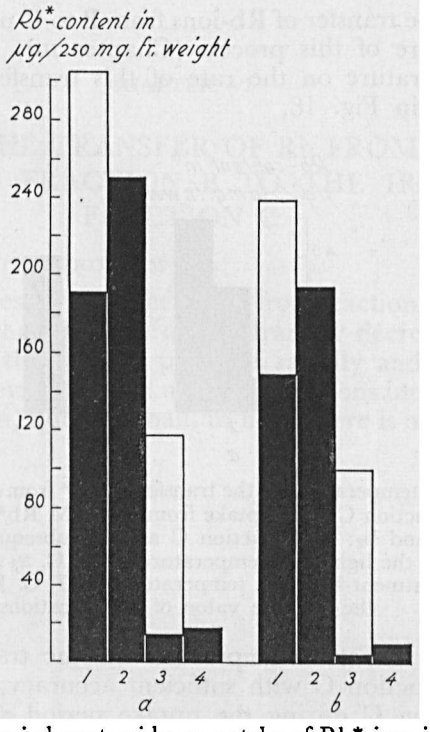

Fig. 17. Effect of monoiodoacetamide on uptake of Rb* ions into the fraction C and on the transfer of Rb* from fraction B to fraction C. a_1 and b_1: Rb* uptake from a .01 M Rb*Cl solution for one hour. Black: Rb* fraction C; white: Rb* fraction B. a_2 and b_2: Rb* fraction C in leaves treated as in a_1 and b_1 and than transferred to deionised water for two hours. a_3 and b_3: Rb* uptake from a .01 M Rb*Cl solution for one hour after a two hours pretreatment with monoiodoacetamide 3×10^{-4} M. a_4 and b_4: Rb* fraction C in leaves treated as in a_3 and b_3 and then transferred to deionised water for two hours. Each column represents the average value of two replications.

During studying the transfer of the Rb-ions of the fraction B to fraction C, the leafstrips must be placed in deionised water to be sure that only ions of the fraction B and not from the medium could reach the fraction C. It appeared that pretreatment of the *Vallisneria* leafstrips with monoiodoacetamide is sufficient to inhibit the subsequent uptake of cations, so that there was no need to supply the monoiodoacetamide to the water in which the leaves remained during the study of the transfer of fraction B to fraction C. However, to obtain an almost complete inhibition of the uptake of cations into the fraction C it was necessary to supply the monoiodoacetamide at least 2 hours before starting the experiment. The results are shown in Fig. 17. As was found in chapter III the size of the fraction B is not affected by the monoiodoacetamide. The present result shows that the transfer of Rb-ions from fraction B to fraction C is strongly inhibited by the monoiodoacetamide.

3. The effect of temperature

The Q_{10} of the transfer of Rb-ions from B to C may give information about the nature of this process (Chapter III). For this reason the effect of temperature on the rate of this transfer was studied. The result is given in Fig. 18.

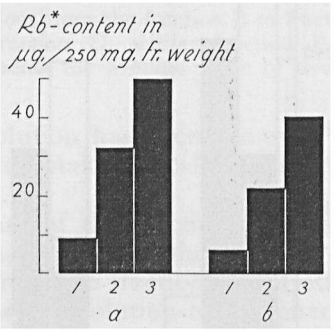

Fig. 18. Effect of temperature on the transfer of Rb* from fraction B to fraction C. a_1 and b_1: Rb* fraction C after uptake from a .01 M Rb*Cl solution in the dark for one hour. a_2 and b_2: Rb* fraction C after a subsequent 30 minutes stay in deionised water in the light at a temperature of 18° C. a_3 and b_3: Rb* fraction C after a similar treatment but at a temperature of 27° C. Each column represents the average value of 4 replications.

To study the effect of temperature on the transfer of Rb-ions of fraction B to fraction C with sufficient accuracy, it was desirable to keep the fraction C during the uptake period as small as possible. In preliminary experiments it was found that the rate of Rb uptake in the fraction C is much lower in the dark than in the light, but that light does not affect the size of the fraction B. For this reason leaf strips were placed in a RbCl solution in the dark for one hour. Moreover, the temperature during the uptake of Rb was rather low, viz. 20° C. After uptake, the strips were rinsed with water and then placed in deionised water at different temperatures.

To obtain a higher degree of accuracy the number of the sets taken for one determination was 6 or 8. During the transfer of Rb from fraction B to fraction C, that is, during the time that the leafstrips were in deionised water, the leafstrips were illuminated.

In Fig. 18a we see that the average uptake of Rb-ions into the fraction C during the uptake period was very low indeed, viz. 9 μg. The transfer from B to C during 30 minutes in water at a temperature of 18° C is 23 μg and at a temperature of 27° C 41 μg. The Q_{10} is 2,1. In Fig. 18b the Q_{10} is 2,15. We may conclude, therefore, that temperature affects the transfer of Rb from fraction B to fraction C strongly, though the temperature quotient is somewhat lower than could be expected for an active transport process. A possible explanation for this discrepancy will be given in chapter VI.

The main conclusion drawn from this chapter is, that the transfer of Rb-ions from fraction B to fraction C as well as the uptake of Rb-ions from the external solution into fraction C depend on metabolism.

CHAPTER VI

THE RATE OF THE TRANSFER OF Rb FROM THE EXCHANGEABLE FRACTION B TO THE IRREVERSIBLE FRACTION C

1. Statement of the problem

Fig. 19, which gives the transfer of Rb from fraction B to fraction C versus time, shows that the rate of this transfer decreases with time.

In the beginning the transfer proceeds rapidly and is of the same magnitude as the previous rate of uptake of Rb-ions into the fraction C from the salt solution, but after half an hour there is a sharp decrease

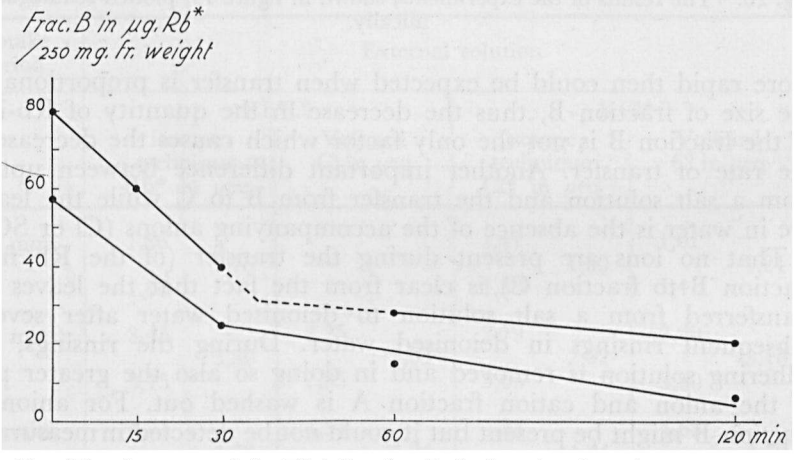

Fig. 19. The decrease of the Rb* fraction B during the time that the leaves are in deionised water. Each point represents the average value of two replications.

in the rate of transfer and even after two hours the fraction B has not been transferred completely to fraction C. This decrease of the rate of uptake occurs only when the leaves are in water. If leaves are bathed in a salt solution the fraction C increases at a constant rate. This will be due to the fact, that when the leaves are in a salt solution the quantity of Rb-ions available for uptake remains almost constant, whereas, when the leaves are in water the size of the fraction B decreases during the transfer to C. When the rate of the transfer is directly proportional to the size of the fraction B, the line which represents the decrease of the Rb fraction B must be a straight line if plotted semilogarithmically (Fig. 20). However, the decrease is

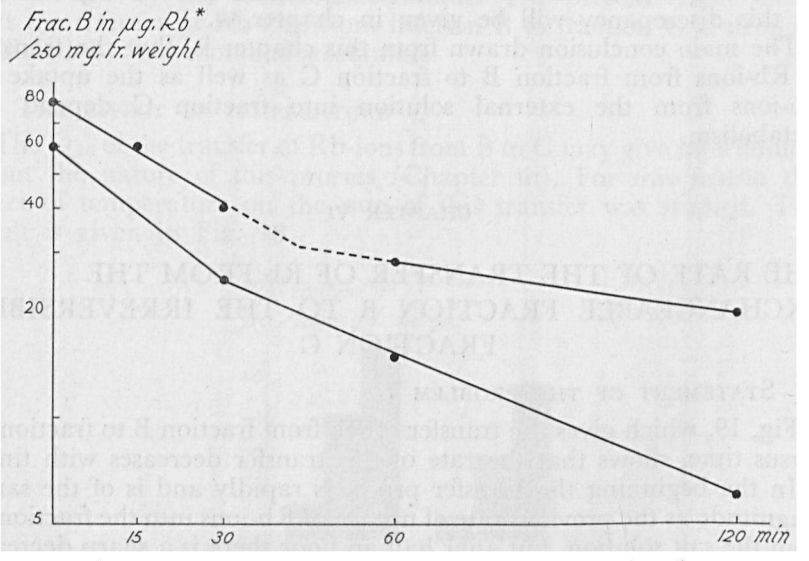

Fig. 20. The results of the experiments, shown in figure 19, plotted semilogarithmically.

more rapid then could be expected when transfer is proportional to the size of fraction B, thus the decrease in the quantity of Rb-ions of the fraction B is not the only factor which causes the decrease in the rate of transfer. Another important difference between uptake from a salt solution and the transfer from B to C while the leaves are in water is the absence of the accompanying anions (Cl or SO_4).

That no ions are present during the transfer (of the Rb from fraction B to fraction C) is clear from the fact that the leaves are transferred from a salt solution to deionised water after several subsequent rinsings in deionised water. During the rinsings, the adhering solution is removed and in doing so also the greater part of the anion and cation fraction A is washed out. For anions a fraction B might be present but it could not be detected in measurable amounts and can, therefore, be neglected (Fig. 5).

The question arises, whether or not the absence of the accom-

panying anion slows down the speed of the transfer of the Rb from fraction B to fraction C.

2. The effect of anions on the rate of the transfer

It is an axiomatic law that the total electrical charge of ions inside plant cells must be equal to zero.

This implicates that either there is an equal uptake of anions and cations by the plant or an exchange of ions which are already present in the plant for ions of equal charge which are taken up. The former possibility was suggested by Hurd and Sutcliffe (1957) and by Hurd (1958) who found that the total uptake of bicarbonate and chloride ions in beetroot disks was equal to the potassium uptake. The second possibility may imply that an excess uptake of added cations is balanced by hydrogen ions which are given off to the external solution in barter for these cations (Jacobson and Ordin, 1954). If accumulation of a cation is only possible when at the same time this cation is joined by an anion, a deficit of accompanying anions might limit the transfer of the Rb from fraction B to fraction C. The number of cations and anions taken up by identical leaves was investigated as follows. A batch of leaves was divided into two equal sets.

One set was bathed in a solution of potassium chloride, the chloride of which contained Cl–36. The other set was bathed in rubidium chloride, the rubidium of which contained Rb–86. The uptake of Cl–36 from the KCl, and the uptake of the Rb–86 from the RbCl were determined as counts per minute, the uptake of chloride from RbCl by Volhard's method. The results are given in table 2 and Fig. 21, calculated as μ equivalents per 250 milligrams

Table 2

Uptake of Rb* from a .008 M Rb*Cl solution and of Cl* from a .008 M KCl* solution

Uptake period	External solution							
	Rb*Cl				KCl*			
	isotope technique Rb* in μeq		Volhard Cl in μeq		isotope technique Cl* in μeq		Volhard Cl in μeq	
60 min.	1,86	1,76			1,41	1,50	1,01	1,21
	1,66				1,58		1,41	
120 min.	3,45	3,30	3,25	3,05	2,79	2,92	2,90	3,10
	3,15		2,85		3,05		3,30	
180 min.	4,71	4,60	4,54	4,24	4,54	4,44	4,62	4,72
	4,50		3,94		4,34		4,83	

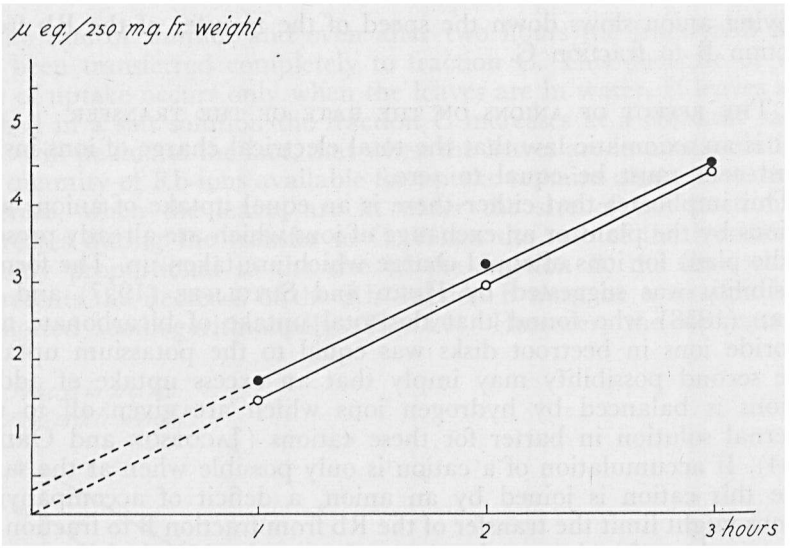

Fig. 21. Course of irreversible rubidium and chloride absorption. ●——● Rb* fraction C after uptake from a .008 MRb*Cl solution; ○——○ Cl* fraction C after uptake from a .008 MKCl* solution. Each point represents the average value of two replications.

fresh weight. Chloride and rubidium ions are taken up in practically equivalent amounts. The uptake of rubidium is slightly higher than that of chloride. If this difference is significant it might be due to the fact that Cl was taken up from a KCl solution. For technical reasons the uptake of labelled rubidium and labelled chloride was not determined from a solution of Rb–86 Cl–36. Another cause for the difference may be that the rubidium fraction B had not completely been removed from the material before C was determined.

A third possibility is that the small excess of Rb-uptake over chloride-uptake was due to the presence of bicarbonate ions. A similar set of experiments was carried out to determine the uptake of sulphate and of rubidium by identical leaves.

Fig. 22 shows that the amounts taken up are not equivalent, the rubidium uptake strongly exceeding the uptake of sulphate-ions. To maintain the theory that the uptake of an anion is coupled with the uptake of a cation, one must assume that here bicarbonate ions join the Rb-ions to compensate for the excess cation uptake and that this can be maintained at a constant speed for several hours. When studying the transfer of Rubidium ions from B to C the presence of bicarbonate ions in the deionised water cannot be prevented.

The bicarbonate concentration may be decreased by lowering the pH of the water, but this procedure would cause the exchange of the Rb-ions from the B fraction against hydrogen ions. For that reason a pH at which a certain concentration of bicarbonate ions exists can not be avoided.

Therefore, the decrease of the rate of transfer of the Rb fraction B to C, which was described in chapter v, might be due to a depletion of the bicarbonate ions. This hypothesis is not supported by the results of Fig. 22, which shows that, under comparable conditions, the excess uptake of Rb over SO_4 may proceed for several hours. This shows, that if this excess uptake is due to bicarbonate ions the

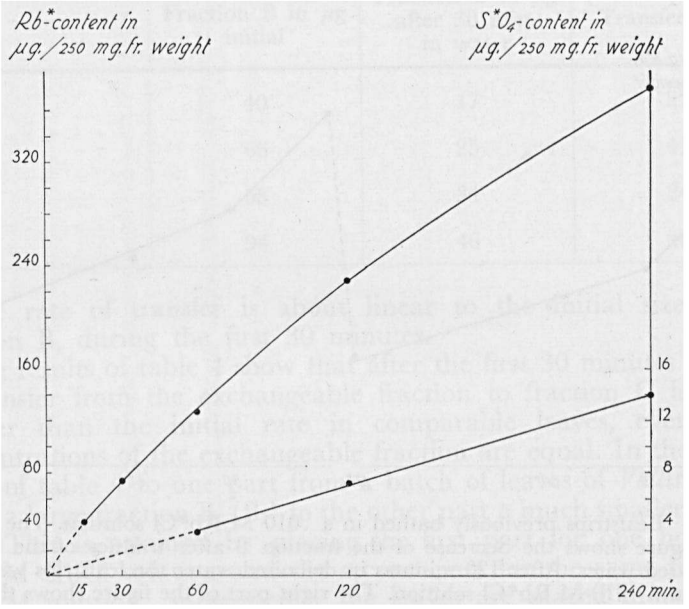

Fig. 22. Course of irreversible rubidium and sulphate absorption. Upper curve Rb* fraction C after uptake from a .004 M $Rb^*_2SO_4$ solution. Lower curve SO_4 fraction C after uptake from a .004 M $K_2SO^*_4$ solution. Each point represents the average value of two replications.

supply of bicarbonate ions allowing the excess uptake is not exhausted within that period.

In the experiments on the transfer of the Rb-ions from B to C, the rate drops sharply after half an hour. It seems unlikely that in the latter experiments the supply of bicarbonate ions would diminish at a different rate from that in the experiment of Fig. 22 and thus, that the rapid decrease of the rate of transfer from B to C is caused by exhaustion of the stock of bicarbonate or of other anions.

It may be supposed that the anions required for the transfer of Rb-ions from B to C, do not form a limited stock, but that they are generated by metabolism. Then, the decrease in rate of the transfer of Rb-ions from B to C might be due to the low rate at which these anions are formed. The following experience makes this supposition rather improbable. After two hours in water the rate of transfer of Rb from B to C has decreased considerably. When at this moment the leafstrips are placed for a second time in a solution of RbCl for one hour, a Rb-fraction B will originate for the second time.

If the rate at which the anions are formed determines the rate of transfer of Rb-ions from B to C, it must be expected that, when the leaves are put in water again, the second B fraction is transferred to C very slowly. However, the opposite is true: the transfer proceeds again at a high initial rate (Fig. 23).

From these data it appears highly unprobable that a decrease in the quantity of the available anions causes the decrease in the rate

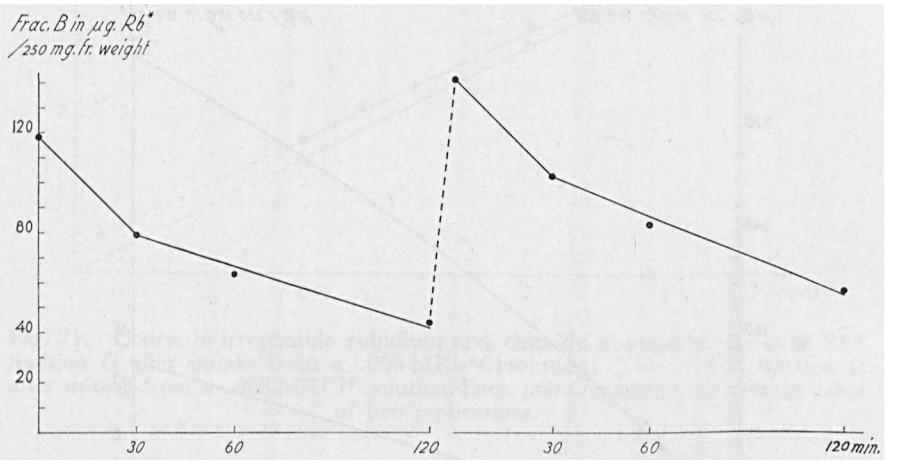

Fig. 23. Leafstrips previously bathed in a .010 M Rb*Cl solution. The left part of the figure shows the decrease of the fraction B after transfer of the leafstrips to deionised water. After 120 minutes in deionised water the leafstrips were again bathed in a .010 M Rb*Cl solution. The right part of the figure shows that, after transfer to deionised water, the fraction B decreases at the same rate as the first time. Each point represents the average value of two replications.

of the transfer. One could object to this that the production of these anions is only possible, when the leaves are bathed in a salt solution and not when the leaves are bathed in deionised water owing to the salt respiration which will doubtlessly rise and may eventually influence the forming of the anions. But it is known from (LUNDEGÅRDH, 1949) that the anion respiration decreases in rate only slowly when plant tissues are transferred from a saltsolution to distilled water.

A period of 120 minutes in deionised water is too short to decrease the rate of the anion respiration to a considerable extent. Summarizing we may conclude that, if anions are required for the transfer of Rb-ions from B to C, their quantity will not become the limiting factor for the rate of this transfer.

3. THE EFFECT OF THE CONCENTRATION OF Rb IN THE FRACTION B ON THE RATE OF TRANSFER FROM B TO C

From the foregoing it is highly probably that the quantity of anions does not determine the rate of transfer of Rb-ions from B to C. For that reason we must assume that it is the number of Rb-ions present

in B that determines this rate. In the experiment of table 3 fractions B of different size were obtained by placing the leaves in a .004 M RbCl or in a .008 M RbCl solution.

TABLE 3

Transfer of Rb*ions from fraction B to fraction C during the first half hour

	Fraction B in μg initial	Fraction B in μg after 30 min. in water	Transfer in μg
Expt 1	40	17	23
	66	25	41
Expt 2	58	34	24
	94	46	48

The rate of transfer is about linear to the initial size of the fraction B, during the first 30 minutes.

The results of table 4 show that after the first 30 minutes the rate of transfer from the exchangeable fraction to fraction C is always smaller than the initial rate in comparable leaves, even if the concentrations of the exchangeable fraction are equal. In the experiment of table 4 to one part from a batch of leaves of *Vallisneria* was given a large fraction B, (B_1) to the other part a much smaller fraction (B_2). This is attained by placing the first part for one hour in a .008 M RbCl solution and the second part for the same time in a .004 M solution. Subsequently the leaves are placed in water and

TABLE 4

Rate of transfer of Rb* ions from fraction B to fraction C.
In each experiment half of the leaves was given a large Rb* fraction B, the other half a much smaller fraction. The leaves were transferred to water and fraction B was determined

Time after transfer of the leaves to water		0′	30′	60′	120′
Expt 1.	Large fraction B_1(μg) . . .	78	66	55	47
	average $-\triangle B_1$/30 min. . .		12	11	4
	small fraction B_2(μg) . . .	48	40		
	$-\triangle B_2$/30 min.		8		
Expt 2.	Large fraction B_1(μg) . . .	66	50	45	44
	average $-\triangle B_1$/30 min. . .		16	5	0,5
	small fraction B_2(μg) . . .	41	31		
	$-\triangle B_2$/30 min.		10		
Expt 3.	Large fraction B_1(μg) . . .	75	55	51	47
	average $-\triangle B_1$/30 min. . .		20	4	2
	small fraction B_2(μg) . . .	44	31		
	$-\triangle B_2$/30 min.		13		

the Rb in fraction C is determined at intervals. Since in water the Rb of fraction B cannot decrease by exchange, the increase of Rb ions in fraction C is equal to the number of ions transferred from B to C ($-\triangle B$). The results are shown in table 4. During the first 30 minutes the $-\triangle B$ is about linear to the size of fraction B at the start of the experiment.

The ratio's of the large and the small fraction $\frac{B_1}{B_2}$ at the start of the experiment are:

$$\frac{78}{48} = 1{,}6 \text{ (Expt 1)}, \quad \frac{66}{41} = 1{,}6 \text{ (Expt 2)}, \quad \frac{75}{44} = 1{,}7 \text{ (Expt 3)}$$

The corresponding ratio's of the transfer from B to C $\frac{\triangle B_1}{\triangle B_2}$ in the first 30 min are:

$$\frac{12}{8} = 1{,}5 \text{ (Expt 1)}, \quad \frac{16}{10} = 1{,}6 \text{ (Expt 2) and } \frac{20}{13} = 1{,}5 \text{ (Expt 3)}.$$

After 60 minutes the large fraction B_1, has decreased to a value only slightly higher than that of the small fraction B_2 at the start of the experiment. If the proportionality between the number of ions in B and the transfer to C had been maintained, in the next 30 minutes, the value of $-\triangle B_1$ should be about equal to that of $-\triangle B_2$ in the first thirty minutes.

However, these values are widely different, viz 4 and 8, 0,5 and 10, 2 and 13. This proves that the Rb ions which are still in B after 60 minutes are transferred at a much smaller rate than Rb-ions at the start of the experiment.

An explanation which may be given for this phenomenon is based on the hypothesis that various types of cells show a difference in the rate of accumulation of ions. That part of the Rb fraction B which is located in the cell-walls of strongly accumulating cells, will be transferred rapidly to C, whereas the part which is located in the cell-walls of the slowly accumulating cells will be transferred very slowly. That the Rb fraction B is located in the cell-walls of all the cells of a leaf is supported by the fact that exchange of Rb in *Potamogeton* can occur readily across the whole leaf (HELDER, unpublished results).

It was shown in chapter v that the Q_{10} of the transfer of Rb-ions from fraction B to fraction C was lower than could be expected for an active transport process. In this chapter it has been shown that the rate of the transfer depends on the size of the Rb fraction B. This fact makes it understandable why the Q_{10} of the tranfer was relatively low. At the high temperature the concentration of the ions of fraction B will decrease at a greater rate than at the low temperature. This decrease will counteract the temperature effect.

CHAPTER VII

THE ROLE OF THE EXCHANGEABLE FRACTION B IN THE IRREVERSIBLE ABSORPTION OF IONS FROM THE EXTERNAL SOLUTION TO C

1. The effect of the size of the fraction B on the rate of increase of the fraction C for Rubidium ions

As has been discussed in chapter VI, the initial rate at which the Rb-ions are transferred from fraction B to fraction C while the leaves are in water, is of the same magnitude as the rate of uptake of Rb-ions into the fraction C when the leaves are bathed in a salt solution. This fact suggests that uptake into the fraction B is a step in the uptake of ions from the medium into the fraction C. But the possibility remains that, normally, ions are transferred directly from A to C. These ions will compete with ions from B for the accumulating mechanism, and, by this, the transfer from B to C may be more or less blocked. The conditions under which this transfer was studied might well have been exceptional, because rubidium ions were absent from the external solution and fraction A.

If one studies Fig. 24, it is striking that the rate of rubidium uptake into the fraction C is nearly independent of the Rb concentration of the external solution, at least within the concentration range which was investigated. Consequently, it is also independent of the size of the fraction B, as this fraction shows an almost linear relationship with the concentration of the external solution. It may be concluded, that, if fraction C is formed by way of the fraction B, a small fraction B is sufficient for a high rate of transfer from B to C.

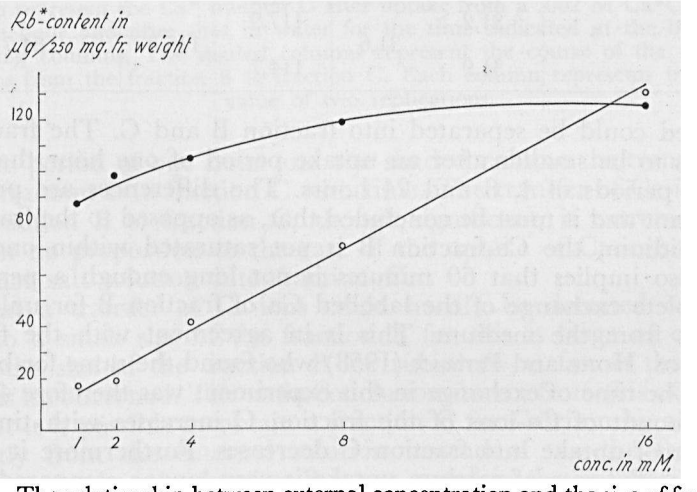

Fig. 24. The relationship between external concentration and the size of fraction B and C for rubidium ions. ●——● the rate of uptake into the fraction C; ○——○ the size of the Rb* fraction B. Each point represents the average value of two replications.

On the contrary, when no rubidium ions are present in the external solution, the initial rate of the transfer of the rubidium from fraction B to fraction C does depend on the size of the fraction B even when this size is great (Chapter VI).

Thus, in contrast to the previous finding this result suggests that a high rate of the transfer is not possible when fraction B is small. This contradiction indicates that fraction B is not intermediate in the uptake of Rb-ions from the external solution into the fraction C.

2. The transfer of Ca-ions from fraction B to fraction C

Other indications that uptake of Rb-ions into the fraction B is not a step in the uptake of cations from the external solution into fraction C, follow from experiments with $CaCl_2$. The method of studying Ca–45 uptake has been described in chapter I in the section on methods. Table 5 shows that, just as with Rb-ions, the Ca-ions

Table 5

Uptake of Ca* from a Ca^*Cl_2 solution

Uptake period	Uptake of Ca* in μg				
	Fraction C		Fraction B+C		Fraction B
1 hour	2,4	2,5	71,5	70,8	68,3
	2,6		70,0		
4 hours	8,6	8,4	85,8	87,5	79,1
	8,2		89,1		
8 hours	15,4	16,0	96,6	94,7	78,7
	16,5		92,7		
24 hours	31,2	31,6	117,9	116,3	84,7
	31,9		114,7		

absorbed could be separated into fraction B and C. The fraction B appears to be smaller after an uptake period of one hour than after uptake periods of 4, 8 and 24 hours. The differences are probably significant and it must be concluded that, as opposed to the fraction B for rubidium, the Ca-fraction B is not saturated within one hour. This also implies that 60 minutes is not long enough a period for a complete exchange of the labelled Ca of fraction B for unlabelled Ca-ions from the medium. This is in agreement with the findings of Briggs, Hope and Pitman (1958) who found the same for beetroot disks. The time of exchange in this experiment was therefore 4 hours. The amount of Ca-ions of the fraction C increases with time, but the rate of uptake into fraction C decreases. Furthermore it can be seen that the rate of calcium uptake is very low as compared to the Rb uptake. Next the transfer of Ca from fraction B to fraction C was examined (Fig. 25).

The white column gives the size of the Ca fraction C after an

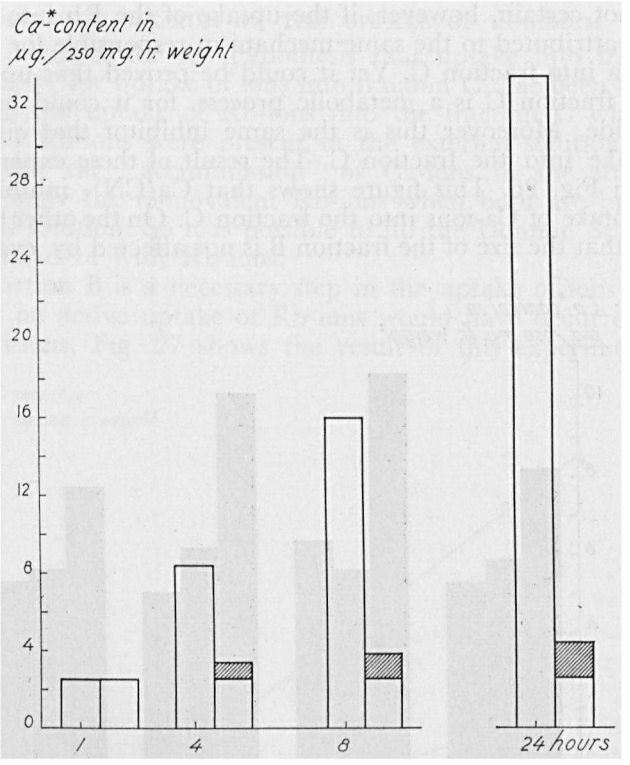

Fig. 25. The course of irreversible calcium absorption and the transfer of calcium from fraction B to fraction C. The left white columns represent the Ca fraction C after uptake from a .002 M Ca^*Cl_2 solution for various periods. The right white column represent the Ca* fraction C after uptake from a .002 M Ca^*Cl_2 solution for one hour and after that in water for the time indicated at the base of the adjoining columns. The shaded columns represent the course of the transfer of Ca*ions from the fraction B to fraction C. Each column represents the average value of two replications.

uptake period of one hour and the shaded column the transfer of Ca from fraction B to fraction C with time. The transfer of Ca-ions from the fraction B to fraction C, though significant, is extremely small and in no proportion to the rate of the uptake of Ca-ions from the external salt solution into fraction C.

That a transfer of Ca-ions could hardly be detected cannot be due to a small size of the fraction B for this is large as compared with the size of the fraction B for rubidium as far as the number of ions is concerned. The main conclusion drawn from this experiment is that it is possible to obtain a fraction C for Calcium by absorption directly from the external solution instead of via the fraction B. This means that the fraction B is not a step in the uptake of Ca-ions into the fraction C, and this supports the hypothesis that also for rubidium the fraction B is not a necessary step in the uptake of Rb-ions into the fraction C.

It is not certain, however, if the uptake of the Rb into fraction C must be attributed to the same mechanism responsible for the uptake of the Ca into fraction C. Yet it could be proved that uptake of Ca into the fraction C is a metabolic process, for it could be inhibited by cyanide. Moreover this is the same inhibitor that inhibited the Rb uptake into the fraction C. The result of these experiments are shown in Fig. 26. This figure shows that $Ca(CN)_2$ inhibits the rate of the uptake of Ca-ions into the fraction C. On the other hand, table 6 shows that the size of the fraction B is not affected by cyanide.

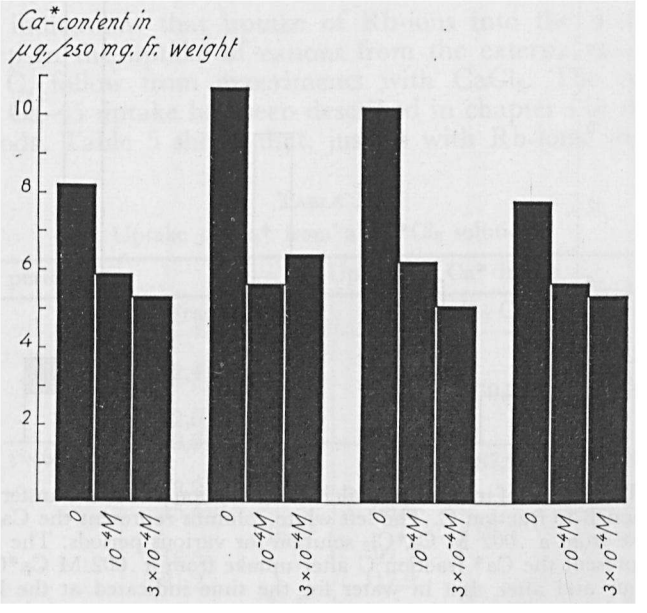

Fig. 26. The effect of 10^{-4} and 3×10^{-4} M $Ca(CN)_2$ on the rate of uptake of Ca ions into the fraction C in four different experiments. Uptake from a .002 M Ca^*Cl_2 solution for 4 hours. Each column represents the average value of two replications.

TABLE 6

The effect of $Ca(CN)_2$ on the size of the Ca* fraction B
1, 2, 3 and 4 give the average Ca fraction B in µg/250 mg fresh weight in separate experiments. Uptake period 4 hours.

External solution	Experiment nr.				
	1	2	3	4	Average
Ca^*Cl_2.002 M	96	94	73	74	84
Ca^*Cl_2.002 M + 10^{-4} M $Ca(CN)_2$	98	94	74	75	85
Ca^*Cl_2.002 M + 3×10^{-4} M $Ca(CN)_2$	90	75	84	78	82

3. The effect of calcium on the uptake of rubidium

Further evidence for the hypothesis that uptake into fraction B is not necessary for uptake of ions into fraction C, has been obtained by studying the uptake of Rb-ions into the fraction C when both Ca-ions and Rb-ions were present in the external solution. It was discussed that the "accumulation" of Ca-ions in the fraction B exceeds strongly the monovalent Rb-ions when both are present in the external solution. So it is possible to fill the Donnan Space with Ca-ions instead of with Rb-ions.

If the fraction B is a necessary step in the uptake of ions into the fraction C, no active uptake of Rb-ions would have occurred under these conditions. Fig. 27 shows the result of this experiment. The

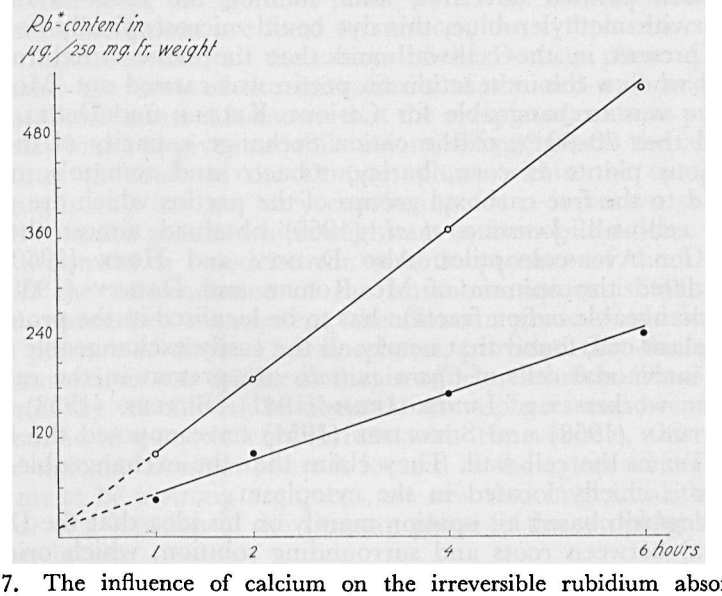

Fig. 27. The influence of calcium on the irreversible rubidium absorption. ○——○ Rb* fraction C after uptake from a .001 M Rb*Cl solution for various periods. ●——● Rb* fraction C after uptake from a .001 M Rb*Cl + .010 M $CaCl_2$ solution. Each point represents the average value of two replications.

concentration in the external solution of Rb was .001 M. Fig. 24 shows that at that concentration the fraction B is very small, namely about 15 μg R b/250 mg fresh weight. The calcium concentration was 10 mM. It was discussed in chapter IV that this will decrease the Rb-ions in B far below 15 μg/250 mg fresh weight. In fact, the amount was so small that it was impossible to determine its actual value. Fig. 27 shows that though Ca decreases the rate of Rb uptake by about 50 %, the rate is still considerable.

Since the rate of transfer from B to C in the absence of ions in the external solution depends on the size of fraction B (table 3) hardly any transfer should have been obtained. From the relatively high uptake of Rb in the presence of calcium it must be concluded that the ions were transferred directly from A to C.

CHAPTER VIII

THE LOCATION OF THE FRACTION B IN THE PLANT CELL

One of the first authors who demonstrated the presence of an exchangeable cation fraction within plant cells and expressed an opinion about its location was DEVEAUX (1896). Deveaux postulated that the exchangeable cation fraction is mainly in the cell-wall where the cations are "electrostatically bound" to the pectins. In this, Deveaux has been supported by the results of various workers MATTSON (1949), KELLER und DEUEL (1957), DAINTY and HOPE (1960), JANSSENS *et al* (1959), MERTZ and LEVITT (1961) etc.

Mattson pointed out that, after staining the roots of different cereals with methylen-blue, this dye could microscopically be shown to be present in the cell-wall and that the same structures were stained when a colour reaction on pectin was carried out. Moreover, this dye was exchangeable for Ca-ions. KELLER und DEUEL (1957) showed that 70–90 % of the cation exchange capacity of the roots of various plants as corn, barley, tobacco and tomato's must be ascribed to the free carboxyl groups of the pectins which are present in the cell-wall. JANSSENS *et al.* (1960) obtained almost the same results for Avea-coleoptiles. Also DAINTY and HOPE (1960) who reconsidered the opinion of MC ROBBIE and DAINTY (1958) that the exchangeable cation fraction has to be localised in the protoplasm of the plant-cell, found that nearly all the easily exchangeable cations in the internodal cells of *Chara australis* are present in the cell-wall.

Other workers e.g. LUNDEGÅRDH (1941), BUTLER (1953), HOPE and PITMAN (1958) and SUTCLIFFE (1954) have opposed the idea of a location in the cell-wall. They claim that the exchangeable cation fraction is chiefly located in the cytoplasm.

Lundegårdh based his opinion mainly on his idea that the Donnan potential between roots and surrounding solution, which originates from the unequal distribution of the cations between the Donnan phase and the surrounding solution, does not exist between cell-wall and surrounding solution but between protoplasm and surrounding solution. As an argument he advances that destruction of the cytoplasmic membrane by heating or by a treatment with alcohol completely alters the picture of the potential change which is induced by dilute acids. While in living cells a change in the concentration of the hydrogen ions of the surrounding solution causes a change in this potential within 0,75 seconds, this change arises only very slowly in cells killed by heating or by alcohol. This, however, does not rule out a location of the Donnan phase in the cell-wall, because pectins are also influenced by heating, (HEINTZE, 1961).

BUTLER (1953) located the fraction A (W.F.S.) partly in the cytoplasm, because a free space of 20 % to 25 % was too high to locate it completely in the cell-wall. This means that ions can pass the plasmalemma by diffusion. If this is true, part of the cation adsorption-exchange might also be located in the cytoplasm, because

the cytoplasm surely contains negatively charged immobile anions.

LEVITT (1959), however, suggested that the W.F.S. of 20–25 % found by Butler is not a pure W.F.S. but partly due to a microscopically thin film of liquid on the surface of the roots.

SUTCLIFFE (1954) found that the exchangeable fraction in beetroot disks decreases when they are rinsed in distilled water at 5° C.

Since, according to Sutcliffe, it was not very likely that, during rinsing, the composition of the cell-wall changes, the effect must be ascribed to the cytoplasm. Again the possibility is not excluded that compounds leached out by the rinsing are responsible for a change in the pectins of the cell-wall.

BRIGGS (1958) who also worked with beetroot disks calculated the relative volume of the Donnan phase at 2,1 %. He calculates that if the immobile anions are fixed to surfaces, the cytoplasmic surface is not sufficient to bind all the cations of the Donnan phase. On the other hand the area of the microfibrils of the cell-wall might be sufficient. But because it is known that the cytoplasm contains proteins which may ionise to give immobile anions, Briggs ignores the cell-wall and locates the bulk of the Donnan phase within the cytoplasm. Briggs arguments are not very convincing for it is known that the cell-wall contains pectins which might act as immobile anions. Briggs second argument is based on the osmotic pressure of the Donnan phase. If the concentration of the immobile anions is 600 μeq/l, and the counterions monovalent, the Donnan phase would have an osmotic pressure of about 11 atmospheres, provided that the activity of the counterions was similar to that in aqueous solution. The osmotic pressure of the vacuole is also about 11 atmospheres and because the cytoplasm is in osmotic equilibrium with the vacuole, this would indicate that the Donnan phase is located in the cytoplasm. Though this seems to be a strong argument, we saw that in general the data in favour of a location of the Donnan phase in the cytoplasm are not very convincing.

It has been demonstrated in chapter v that the Rb-ions of the fraction B can be transferred to fraction C and that this process can be inhibited by monoiodoacetamide.

Data about the location of the fraction B will, therefore, give information about the location of the accumulation mechanism by which ions are transferred from B to C. Since in literature opinions differ on the site of the exchangeable cation fraction it seemed worth-while to find out the location of the fraction B in *Vallisneria* leaves. As the establishment of the exchangeable fraction is completely independent of metabolic activity, it could be expected that the Rb fraction B, if situated in the cell-wall, will also be formed when the cell-wall is separated from the cell.

In the following way the exchangeable ions in isolated cell-wall material were determined. In order to separate the cell-wall from the cytoplasm and vacuole, *Vallisneria* leafstrips were homogenised for 3 minutes in a Bühler homogeniser at a speed of 25000 rotations per minute. Next, the cell-wall material was precipitated by centri-

fuging (100 g) and washed thoroughly. It could be shown microscopically that after centrifuging about 90 % of the cells are optically empty. The rest of the cells were not destroyed by the homogeniser. The percentage of organically bound nitrogen in the precipitate as determined by the micro-Kjeldahl method, (LOOMIS and SHULL, 1937) amounted to 10 % of that of the living leaf. This is in good agreement with the percentage of the non emptied intact cells present in the isolated cell-wall material.

The cell-wall material of a number of sets was suspended in a Rb*Cl solution and precipitated after 60 minutes. The cell-wall samples were next rinsed in deionised water or bathed in an unlabelled RbCl solution. From this the fraction (B + C) and C could be determined as described in chapter I, for intact leaves. During the isolation of the cell-wall material, a fraction, equivalent to four leafstrips each measuring 2,5 × 4 mm, was always lost.

For this a correction has been made. Besides a correction for the loss of cell-wall material also a correction was made for the percentage of intact cells in the precipitate.

When W represents the actual % of Rb* in the cell-wall and E is the % experimentally found

$$W = \frac{10}{9} (E - 10).$$

The results are shown in table 7.

TABLE 7

Rb* uptake by the isolated cell-walls from 4 sets Vallisneria leafstrips. Fraction B in percents of the exchangeable fraction of intact leaves.

Data obtained in different experiments	Average	After correction for 0,5 set	After correction for intact cells
36			
47			
50			
40	41	47	41
40			
23			
45			
49			

From these experiments the conclusion can be drawn that only 40 % of the fraction B can be located in the cell-walls. This would mean that the other part has to be located somewhere in the cytoplasm, if at least the method is completely reliable. However, in chapter IV it has already been discussed that it is difficult to determine the maximal size of the exchangeable cation fraction by means of a monovalent cation. If other monovalent cations and especially divalent cations are present they will interfere strongly with the Rb-ions. The degree of competition depends on the concentration of the interfering cations.

For technical reasons it was not possible to keep the concentration of these interfering cations constant neither during the determination of the size of the fraction B in intact cells nor during the determination of the fraction B in isolated cell-wall material. The volume of the salt solution in which the cell-wall fraction was suspended, was much smaller than the volume in which the intact cells were bathed. Thus, an equal quantity of exchanged ions from the material would attain a higher concentration in the former solution.

It is conceivable, therefore, that the measured low exchange capacity of the cell-wall for Rb is due to a higher concentration of interfering ions. Though this has not been experimentally checked, it throws doubt upon the validity of the value arrived at. A trivalent ion would, at least if it could enter the Donnan Space, practically not be influenced by interfering monovalent and divalent cations because of its much higher charge. That is why the trivalent Lanthanum ion has been used to ascertain the exchange capacity of the cell-wall.

In preliminary experiments with unlabelled Lanthanum it was established that Lanthanum can enter the Donnan Space for it was found that it could remove Rb from fraction B by exchange. However, if the Donnan Space had previously been filled with Lanthanum it is very difficult to exchange the Lanthanum ions for Rb ions: it was found that a fraction B did not arise when these Lanthanum pretreated leaves were bathed in a RbCl solution at a high concentration (.01 M). for as long as one hour. Lanthanum is not injurious to *Vallisneria* leaves, provided it is not administered in too high a concentration and for too long a time. It should be noticed that the criterion for this, viz. infiltration of the intercellular spaces, is a very sensitive one. The determination of the percentage of the La fraction B that is present in the isolated cell-wall was carried out in a way similar to that for Rb. First the La fraction B in intact cells was determined.

Then the quantity of Lanthanum in the isolated walls of an equal number of leaf strips was measured. The uptake of labelled Lanthanum in intact leafcells (black dots) and the decrease in the leaves of labelled Lanthanum by exchange against unlabelled Lanthanum are shown in Fig. 28 (circles). After 24 hours the uptake of Lanthanum was still going on.

The small rate indicates that it had nearly come to a stop. The curves represent the sum of the fraction B and C. It must be assumed that fraction C is small. Firstly, the exchange curve (open circles) shows that after 19 hours a small quantity of labelled Lanthanum remained in the leaves. By that time the exchange was still proceeding, but the experiment had to be cut short because infiltration of the intercellular spaces began. Thus, only a part of the small quantity of labelled ions left after 19 hours will have belonged to fraction C. A second reason for assuming that the fraction C is small for Lanthanum is the fact that this fraction is small for the bivalent Ca. It is very likely that with trivalent Lanthanum fraction C is still

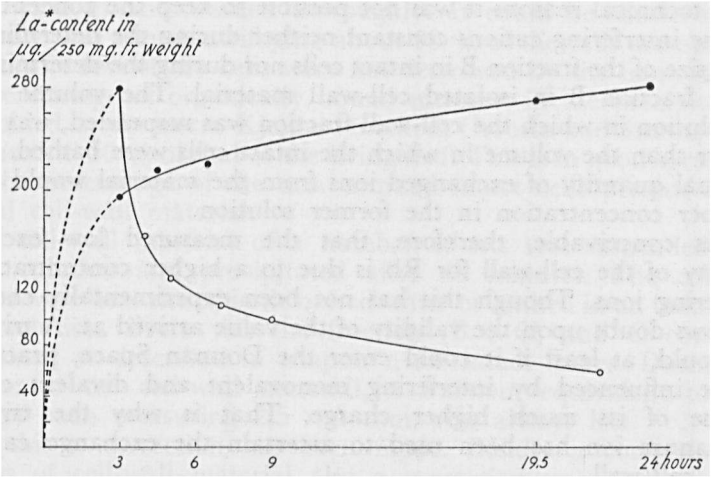

Fig. 28. The course of lanthanum absorption and exchange in intact leaves. ●——● La uptake from a .00186 M La^*Cl_3 solution after various periods. ○——○ labelled La content after exchange of the La* absorbed within the first three hours for unlabelled La. Each point represents the average value of two replications.

smaller. Therefore, it can be safely assumed that the whole uptake of La by Vallisneria leaves is adsorption-exchange (Fraction B).

The method of preparing the cell-wall material was the same as described above for Rb except that homogenizing and washing was carried out at a rather low temperature (0° C–10° C) and in the presence of an acetate buffer pH 4,2. (Jansen, *et al.*) in order to prevent a possible deesterification of the pectins in the cell-wall. According to KELLER and DEUEL (1957) these pectins are responsible for the adsorption of the greater part of the fraction B. In the course of the experiments it was found that neither temperature nor the use of an acetate buffer had any influence on the size of the Lanthanum fraction B, i.e. on the exchange capacity. The washed precipitate

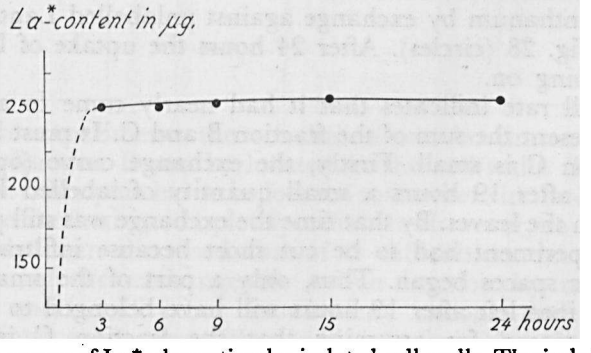

Fig. 29. The course of La* absorption by isolated cell-walls. The isolated cell-wall material is obtained from 1 g fresh weight leaf material. Each point represents the average value of two replications.

was next suspended in a labelled .00186 M $LaCl_3$ solution and the size of the fraction B determined after different incubation periods. The results are shown in Fig. 29. It appears that after an incubation period of 3 hours the size of the La fraction B in the cell-wall homogenate remains constant.

Thus it was sufficient to bathe cell-wall homogenate during three hours in $LaCl_3$, whereas intact cells must be in contact with the La ions during 24 hours. The results are given in table 8.

The percentage of Lanthanum found in the isolated cell-wall material increases with the number of leaves used. In Fig. 30 this percentage has been plotted against the reciprocal of the number of leaves used in each experiment. The points fit a straight line that can be described by the equation. $Y = K - \frac{A}{x}$.

In this equation Y stands for the amount of Lanthanum found in isolated cell-walls as percentage of Lanthanum in intact leaves, x for the number of leaves used; K and A are constants.

Presumably, A represents a definite quantity of cell-wall material

TABLE 8

La* uptake by the isolated cell-walls of Vallisneria leaves in percents of the uptake of La* by intact leaves

Number of sets	La* uptake in percents of the La* uptake by intact leaves
2	76
3	84
4	86
6	89
8	91

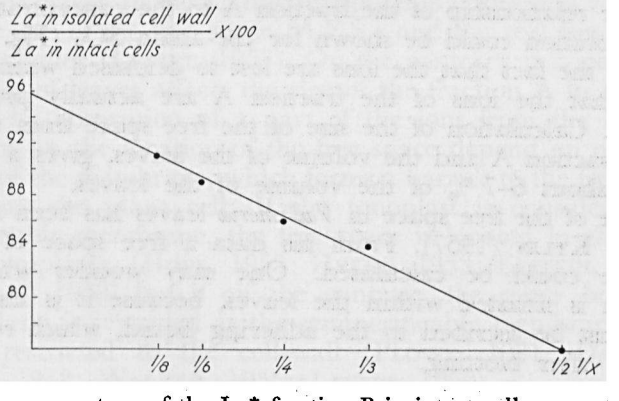

Fig. 30. The percentage of the La* fraction B in intact cells present in isolated cell-walls in dependency of the amount of leaf material taken for the preparation of cell-wall material. × represents the number of sets. Each point represents the average value of two replications.

that is lost during the preparation independent of the number of leaves used. When the number of leaves used is increased the factor $\frac{A}{x}$ decreases and the value for the percentage of Lanthanum in the cell-wall approaches to K. The intercept of the curve and the ordinate gives the value of K when an infinite number of leaves would have been used in the preparation of the cell-wall material. This value amounts to 96 %. This means that practically the whole Lanthanum fraction B is present in the cell-walls. One may wonder whether the same holds for the Rb-ions. We saw that it was only possible to demonstrate that at most 40 % of the Rb fraction B is in the isolated cell-wall. However, it was possible to exchange the whole Rb fraction B for La ions. From the result follows that if the La fraction B is located in the cell-wall, the Rb fraction B too must be located in the cell-wall.

2. The location of the Rb fraction A and its nature

In chapter II it has been shown that a fraction A originates in the cells of *Vallisneria* leaves, when these leaves are bathed in a solution of RbCl. The fraction A is given off again, when the leaves are next bathed in deionised water. The ions of the fraction A are therefore present in a compartment into which they easily enter by diffusion. If this compartment is identical to what usually is called a free space, the concentration of the ions in this compartment is the same as in the surrounding solution, and the size of the fraction A must be linearly proportionally to the concentration of the external solution after establishing of the equilibrium. However, it is not possible to prove this experimentally with Rb-ions. The size of the Rb fraction A was small as compared with the Rb fraction B and as has been discussed in chapter 3, this makes it difficult to determine the Rb fraction A with sufficient accuracy.

A linear relationship of the fraction A to the concentration of the external solution could be shown for the anion SO_4 (Fig. 31). This result and the fact that the ions are lost to deionised water, indicate strongly that the ions of the fraction A are actually present in a free space. Calculation of the size of the free space from the size of the SO_4 fraction A and the volume of the leaves, gives a free space equal to about 6–7 % of the volume of the leaves.

The size of the free space in *Vallisneria* leaves has been also determined by Kylin (1957). From his data a free space of the same magnitude could be calculated. One may wonder whether the fraction A is situated within the leaves, because it is also possible that it must be ascribed to the adhering liquid, which remains on the leaves after blotting.

Though the adhering salt solution surely screens an exact determination of the size of the fraction A, the possibility can be excluded that it constitutes the total fraction A, as the SO_4 fraction A reaches its maximum size only after 5–10 minutes (Kylin, 1957).

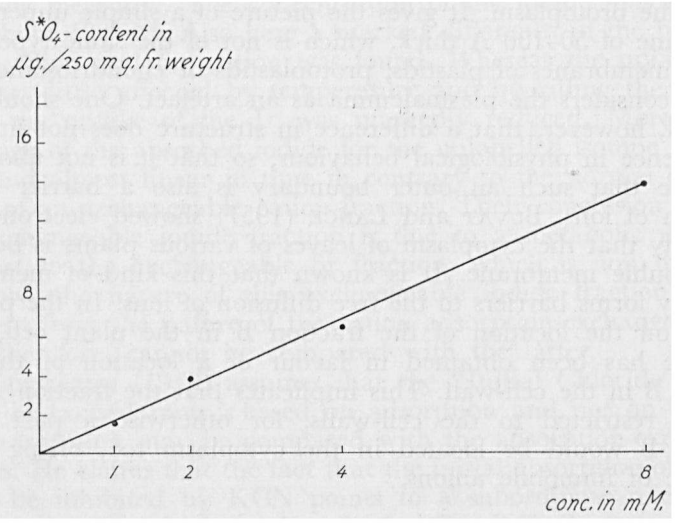

Fig. 31. The size of the S^*O_4 fraction A after an one hour uptake from various concentrations of $K_2S^*O_4$. Each point represents the average value of four replications.

KYLIN (1960) obtained still another argument indicating that the fraction A has not to be considered as an artefact. He actually showed that the moss *Thuidium*, which has a surface area of about 700 cm^2/g fresh weight has a free space which is larger than that of the moss *Plagiothecium*, though this moss has a surface area of about 1000 cm^2/g fresh weight. The free space of roots, called A.F.S. by HOPE and STEVENS (1952) is considerably higher than that of *Vallisneria* leaves and lies between 20 and 33 % of the root volume (BUTLER, 1953; EPSTEIN, 1955). Because the quotient $\frac{\text{surface}}{\text{volume}}$ is higher in roots than in *Vallisneria* leaves, in roots a microscopic thin liquid film will strongly screen the size of the free space (A.F.S.) and the real free space will be much smaller (LEVITT, 1957). On the contrary it is possible that the blotting of leaves affects the size of the fraction A in the reverse direction, by absorption of a part of the ions from the free space.

Opinions on the location of the free space depend on opinions on the place of the membrane, which forms a barrier to the free diffusion of ions into the plant cell. If the tonoplast is considered as the semipermeable membrane, the free space is located in the cell-wall and the cytoplasm, (HOPE, 1953; HYLMÖ, 1953; EPSTEIN, 1956) and BRIGGS *et al.* 1958) but if on the contrary the outer boundary of the cytoplasm is regarded as the semipermeable membrane, the free space is restricted to the cell-wall (PLOWE, 1931; DAVSON and DANIELLI, 1943; WALKER, 1955; LEVITT, 1960).

With the aid of the electron microscope it has been shown by SJÖSTRAND (1954) that in meristematic cells there is a structural difference between the outer boundary of the protoplasm and the

rest of the protoplasm. It gives the picture of a simple unperforated membrane of 50–100 A thick, which is not of the same type as the double membranes of plastids, protoplastids or chondriosomes. This worker considers the plasmalemma as an artefact. One should keep in mind, however, that a difference in structure does not implicate a difference in physiological behaviour, so that it is not allowed to conclude that such an outer boundary is also a barrier to free diffusion of ions. BUVAT and LANCE (1957) showed electron-microscopically that the cytoplasm of leaves of various plants is bordered by a double membrane. It is known that this kind of membranes generally forms barriers to the free diffusion of ions. In the previous section on the location of the fraction B in the plant cell, much evidence has been obtained in favour of a location of the total fraction B in the cell-wall. This implicates that the fraction A must also be restricted to the cell-walls, for otherwise a part of the fraction B would be located in the cytoplasm too, owing to the presence of immobile anions.

CHAPTER IX

GENERAL DISCUSSION

THE PRESENCE OF AN EXCHANGEABLE ANION FRACTION

In chapter II it has been shown that an anion fraction B, if present, must be very small. According to HOPE (1953) this is intelligible because at normal pH's the acid dissociation of amphoteric groups strongly predominates in plant cells. Also in literature there is much evidence for the existence of an exchangeable cation fraction in plant tissues but little for an exchangeable anion fraction (KYLIN and HYLMÖ, 1957; LEGGETT and EPSTEIN, 1956). This is in accordance with the present experiments. Yet there are some reports of an exchangeable anion fraction.

EPSTEIN (1955) found in barley roots a small labile bound SO_4 fraction. Selenate ions proved to compete with the SO_4 ions for the binding-sites and for this reason, this SO_4 fraction has been considered to be due to adsorption-exchange (LATIES, 1959). Because it could be washed out in deionised water, we must accept that it is exchanged either for the hydroxyl ions, which are present in a very small concentration or for other anions in the medium. These anions may have diffused out of the plant tissue.

OVERSTREET and JACOBSON (1946) found in barley roots an exchangeable phosphate fraction but there was a marked difference in behaviour between the exchangeable Rb fraction and the exchangeable phosphate fraction in regard to their exchange for the unlabelled isotope. Whereas the Rb ions were efficiently removed, the phosphate ions were only slowly removed at a constant rate.

They placed the roots at a temperature of zero degree centigrade in a salt solution of the carrier-free iodine. By this the specific activity was very high and uptake and exchange of very small fractions

could be measured. The concentration of the iodide ions was very low, about 10^{-9} M. Also here a marked difference in the behaviour of the anion and the cation was found. Whereas the uptake of Sr was very little affected by temperature and by killing the roots by ether, the uptake of the I^- was markedly reduced. Moreover the exchange of the absorbed iodide for the unlabelled isotope was very slow and almost linear to time in contrary to the normal exchange curve of an exchangeable cation fraction. Their conclusion was that the exchangeable iodide fraction is due to a metabolic uptake in contrast to the exchangeable Sr fraction, which is non metabolic. Anyhow, the nature of this exchangeable iodide fraction is quite different from the nature of the cation adsorption-exchange fraction and, therefore, cannot be compared with the latter.

LUNDEGÅRDH (1958) assumes that the "Initial Chloride Uptake" found in excised roots is based on adsorption and not on diffusion. This adsorption may be compared with the adsorption-exchange of cations. He claims that the fact that the initial absorption of chloride could be inhibited by KCN points to a subordinate role of pure diffusion in the initial uptake of salts. Lundegårdh, however, only determines differences in the concentration of the external solution before and after the uptake period. In this way adsorption-exchange cannot be demonstrated and experimental evidence for his conception was not obtained.

CARRIERS

As has already been mentioned in chapter I one of the theories about the mechanism of ion absorption by living cells is the carrier theory. This theory starts from the hypothesis that the ions are bound to some movable compound of the cytoplasm, which can pass a membrane impermeable to free ions. It may be asked whether or not the exchange sites to which the cations of fraction B are attached are identical with these carriers. By the experiments of several investigators EPSTEIN and HAGEN (1952), SCOTT and HAYWARD (1954), EPSTEIN and LEGGETT (1954), it has been shown that a very specific competition exists between ions of the same charge and between different groups of ions. Therefore, it is believed that special carriers react with particular ions. However, in *Vallisneria* there proved to be practically no specificity of the exchange sites for monovalent cations (chapter IV). This result is not in favour of the opinion that the exchange sites of fraction B are carriers in the above mentioned sense (LUNDEGÅRDH 1958).

A second objection is that the number of exchange sites is very large. This is in contrast with the generally accepted idea that carriers are present in very small amounts (HAGEN and HOPKINS, 1955; HAGEN, LEGGETT and JACKSON, 1957). The third objection is that the amount of anion carrier would be very small as opposed to the amount of the cation carriers. The most convincing proof against the supposition was obtained in the present experiments in which it

was demonstrated that the adsorption exchange (Fraction B) is not a necessary step in the metabolic uptake of the cations.

We may, therefore, safely assume that the adsorption-exchange in *Vallisneria* has nothing to do with reversible binding to carriers as described by the supporters of the carrier theory.

The location of the exchangeable fraction

It has been concluded in chapter VIII that 96 percent of the La fraction B is located in the cell-wall. However, in calculating the percentage of the La fraction B in the cell-wall, the starting point was that the whole uptake of La-ions in *Vallisneria* leaves is non-metabolic, viz. fraction B. Though it has been discussed in chapter VIII that a metabolic dependent La uptake, if present, must be very small, the possibility that a small metabolic dependent La uptake is present, can not be fully excluded. This possible error would decrease the calculated percentage of the La fraction B in the cell-wall. On the other hand we have seen that the La uptake in the living cell has not completely stopped after 24 hours. This may affect the result in the opposite direction. Both errors are small and they will not influence the result very much. Another objection against the conclusion that the La fraction B is in the cell-wall, may be based on the possibility that by homogenizing the tissue, the cell-wall is not completely separated from the cytoplasm. A measure for cytoplasm in the homogenate is its nitrogen content. The cytoplasm in the cell-wall after preparation is, at least partly, present in intact cells. These cells constitute a certain percentage of the total number originally present in the leaves. This percentage was determined by counting. It was approximately equal to the percentage of the nitrogen of the intact leaves that had remained in the cell-wall. This indicates that apart from the intact cells, little cytoplasm was present.

A second indication that the La fraction B is really located in the cell-wall, follows from the result, that the La fraction B in isolated cell-wall from plasmolysed cells has the same size as the La fraction B in isolated cell-wall from unplasmolysed cells. It is very unlikely that in plasmolysed cells the whole surface layer of the cytoplasm adheres to the cell-wall. Therefore, it is concluded that the exchange sites for the La ions are located in the cell-wall.

The transfer of Ca from fraction B to fraction C

In chapter VII it has been shown that the Ca-ions of the fraction B are hardly transferred to fraction C, whereas a metabolic Ca absorption occurred if the leaves were bathed in a salt solution of $CaCl_2$. This metabolic uptake of Ca into fraction C could be maintained for at least 24 hours. It may be asked why transfer of Ca from B to C does not take place, whereas Rb is readily transferred. As ions can be obtained from fraction B by exchange for other ions only, the transfer from B to C must depend upon exchange. It has been discussed in chapter IV that exchange of divalent cations of the D.F.S. for

monovalent cations is difficult, whereas the monovalent rubidium ions are readily exchanged for other monovalent ions. If this is true we may conclude that the transfer of ions from fraction B to fraction C depends upon exchange of these ions for monovalent cations (probably hydrogen ions) from the cell.

THE TRANSFER OF CA FROM FRACTION A TO FRACTION C

It was concluded from the experiments in chapter VII on the Ca uptake that the metabolic Ca uptake into the fraction C occurs directly from the external solution (fraction A) and not via the Ca fraction B. This has been considered to be a strong indication that the same holds for the Rb uptake into the fraction C.

One may wonder whether this is justified. In the following respect uptake of rubidium is similar to the uptake of calcium. The uptake into C of both ions is an active process, which may be inhibited by the same metabolic poisons. The fraction B of both cations is present in the cell-walls, since both may be exchanged for lanthanum and since 96 % of the lanthanum fraction B is located in the cell-walls. Neither a calcium fraction B nor a rubidium fraction B has been demonstrated to be present in the cytoplasm. This means that the active uptake into C takes place at the outer boundary of the cytoplasm. The only difference is that rubidium may be transferred from A to C and from B to C, whereas calcium is only transferred from A to C. There is no reason whatsoever to assume that a calcium fraction A may penetrate beyond the outer layer into the cytoplasm whereas a rubidium fraction A would stop at that layer. The fate of the two cations in the cell may be different, but it is not relevant to the question considered here. Whereas the Rb-ions are probably mainly accumulated into the vacuole (A. VAN SCHREVEN and A. VAN DER MOLEN, ARISZ in 1943, 1956) the Ca presumably remains in the cytoplasm (MAZIA, 1938), though it is also known of Ca ions that they may accumulated in the vacuole (CHASSON and LEVITT, 1957). We can therefore safely assume that the conclusion drawn from the experiments with $CaCl_2$, that the exchangeable fraction is not necessary for irreversible uptake, also holds for the Rb uptake into the fraction C.

THE LOCATION OF THE METABOLIC UPTAKE PROCESS IN THE CELL

From previous researches it has appeared that the uptake of chloride into the cytoplasm of *Vallisneria* leaves is an active process, using energy available in the cell or supplied when exposed to light (ARISZ, 1947, 1952, 1956). In chapter III it has been shown that the rate of the Rb uptake into the fraction C can strongly be inhibited by inhibitors of metabolic processes.

Moreover it was shown that the rate of uptake was dependent on temperature. From these results it may be concluded that the cation uptake into the fraction C is also only possible by an "active" accumulation mechanism. However, according to LUNDEGÅRDH (1945) the possibility remains that the rate of the cation accumulation in

cells is only inhibited by such factors as poisons, temperature etc., because the uptake of the accompanying anion is inhibited by these factors. The cation accumulation in it self may be a non-metabolic process, so that the cations are dragged passively with the anions, in order to maintain electrical neutrality within the cell. Whether the Rb-ions are passively dragged into the cells by anions which are taken up actively, or taken up actively is irrelevant to the problem discussed here.

In chapter v it was shown that not only the uptake of Rb into C is a metabolic process but also the transfer from B to C. It could be inhibited by monoiodoacetamide and it had a high temperature quotient. During the transfer of the Rb from fraction B to fraction C no added anions are available to join the Rb-ions. A fraction A has been washed out in the deionised water (chapter v) and for the anion a fraction B does not exist in measurable amounts (chapter II). Because in several experiments the pH of the deionised water was held at a pH 5, it is unlikely that measurable amounts of bicarbonate ions, that might join the Rb ions during the transfer to the fraction C, were present in the deionised water. These findings are strong indications that an uptake of cations exists which is independent of the uptake of anions. It follows that the cation uptake can directly be dependent on metabolism and that the outer boundary of the cytoplasm forms the barrier for a free diffusion of ions, which can only be passed at the expense of energy.

ARISZ demonstrated (1956) that cyanide has not a direct inhibiting influence on the secretion into the vacuole, but that it inhibits a process by which ions are actively absorbed into the cytoplasm. This shows that two different mechanism for the metabolic uptake of chloride are present in *Vallisneria* leaves. Arisz located one mechanism in the tonoplast and the other one somewhere in the cytoplasm. The present experiments do not permit any conclusion with regard to the accumulation mechanisms located at the tonoplast, but they furnish data on the existence of a mechanism regulating the absorption into the cytoplasm and moreover they allow conclusion about the place where this mechanism is located.

ROBERTSON *c.s.* (1955) assume that the accumulation mechanism is not located in the plasmalemma but in the outer boundary of mitochondria. According to ROBERTSON (1957) it is possible that these mitochondria move around by the protoplasmic streaming in the cell and that many of them frequently come into contact with the surface of the vacuole. It would be equally possible that they come into contact with the cell-wall, pick up ions here and lose them to the vacuole. However, it is known that in *Vallisneria* leaves the protoplasmic streaming comes to a standstill (JAGER, 1958) under conditions of high metabolic uptake and is not restored for the first few hours after bringing the leaves to deionised water. Thus a transfer of ions from wall to cytoplasm by means of mitochondria is not very likely. But even when it should take place, the first accumulation mechanism must be located at the boundary of cytoplasm and cell-wall.

SUMMARY

1. Cations absorbed by *Vallisneria* leaves could be separated into three fractions, viz:
1. a fraction which was washed out in deionised water (fraction A)
2. a fraction which can be removed by exchange (fraction B)
3. a fraction which can be neither washed out nor exchanged.

2. Absorbed anions could be separated into two fractions only, viz: a fraction A and a fraction C; an exchangeable fraction being absent (chapter II).

3. The formation of the exchangeable fraction B depends on the physico-chemical and that of the fraction C on the biochemical properties of the plant (chapter III).

4. It is concluded that a Donnan equilibrium determines the exchangeable fraction B (chapter IV).

5. The transfer of rubidium ions from the exchangeable fraction B to fraction C is strongly affected by temperature and is inhibited by monoiodoacetamide (chapter V).

6. Of each number of rubidium ions accumulated as fraction B (exchangeable fraction) one part is more readily transferred to C than the rest. The rate of transfer of the first part is approximately linear to the number of ions present in B. The second portion is transferred at a much lower rate (chapter VI).

7. In contrast to exchangeable rubidium-ions, exchangeable calcium-ions of the fraction B are practically not transferred to fraction C, though Ca-ions are taken up in C from the external solution. This uptake depends on metabolism. It is concluded that adsorption-exchange in fraction B does not constitute a necessary link in the uptake of cations from the external solution into fraction C (chapter VII).

8. It is made probable that the exchangeable fraction (fraction B) is located in the cell-wall, and that the metabolic process which accumulates cations into the fraction C is located at the outer boundary of the cytoplasm. (Chapter VIII and General Discussion).

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