Kinetics of Rb Absorption by Excised Barley Roots under Changing Rb Concentrations. II. An Interpretation of Kinetic Data

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In studies of salt absorption by plant roots, the rate of uptake is usually expressed as a simple function of concentration. From data plotted in such a manner, however, only limited interpretation can be made. Judicious use of the Michaelis-Menten equation, which is based on mass action expression of steady state kinetics, makes it possible to analyze still further certain aspects of ion absorption; but, the limitations of this concept must be recognized. Among the first to use it in salt absorption by plant roots were Epstein and Hagen (3) who applied it to elucidate the nature of the inhibition of Rb uptake by other cations.

The Michaelis-Menten equation can be employed to give a linear relationship between absorption rate and concentration over a limited concentration range. Over a wide range in concentrations, however, a nonlinear relationship is observed. This has been interpreted as indicating the participation of 2 sites or processes in cation uptake (5).

In this work, it was found that the rate of Rb uptake (v) by barley and mung bean roots can be expressed as a function of concentration (C) over a wide range in concentrations, from about 10^{-6} to 5 \times 10^{-3} \text{ M}, by an equation similar to the Freundlich adsorption isotherm. Thus,

\[ v = kC^n \]  

I

or

\[ \log v = n \log C + \log k \]  

II

where n and k are parameters. An expression of this form appears to fit data obtained over a limited range by earlier workers (12, 13).

Equation I has been used in this paper to derive an expression which relates the concentration to time when the concentration is changing during absorption. To do this, equation I is rewritten so that

\[ v = -\frac{dC}{dt} = kC^n \]  

where \( \frac{dC}{dt} \) is the rate of concentration change or the amount of Rb absorbed during a short interval. Integration of equation Ib in the region of C gives

\[ C^{(1-n)} = -k't + C_o^{(1-n)} \]  

II

where \( C_o \) is the concentration at time zero.

Materials and Methods

Barley seeds (Hordeum vulgare L., var. Atlas 46) were germinated and the seedlings grown in darkness with roots in aerated, 4 \times 10^{-4} \text{ M} \text{ CaSO}_4 solution at 22\degree. When the seedlings were 4 days old, they were selected for uniformity in size. Their roots were cut off with a razor blade about 1 cm below the seed. The roots were washed several times with deionized water. Small amounts of roots were quickly blotted with filter paper and weighed. From 0.2 to 1.5 g of roots were placed in 20 to 50 ml of absorption medium, the amount and volume depending upon the Rb concentration. At low Rb concentrations roots were prewashed for about 20 minutes with 10^{-3} \text{ M} Tris to remove external Ca. Ca inhibited the uptake of Rb at low concentrations. Distilled water redistilled from a glass still was used to make up Rb solutions of low concentrations. To avoid the inhibitory effects of H ions, 10^{-3} \text{ M} Tris was used as a buffer. The initial pH was 8.3, and it decreased...
slightly during the course of an experiment. No adverse effect by the buffer on Rb absorption was observed. The length of a run ranged from 30 minutes to about 2 hours, depending upon the Rb concentration. Rb was added as the chloride. The initial Rb concentrations ranged from $3 \times 10^{-3}$ M down to $10^{-6}$ M. Radioactive Rb$^{86}$ was used as a tracer. Non-specific adsorption of Rb ions on sites other than Rb-absorbing sites was corrected for by taking a sample of the solution 1 or 2 minutes after beginning of an experiment. It is assumed that this adsorption is rapid and is essentially over in about 1 minute. Thereafter, samples were taken periodically and analyzed for radioactivity to follow the changes in Rb concentration with time. The volume of the samples was kept small to reduce volume changes. It ranged from 0.2 to 0.5 ml. The solution was aerated vigorously, and its temperature was maintained at 22° by a water bath. Some experiments were carried out at lower temperatures. Polyethylene apparatus was used to minimize adsorption of Rb.

In some experiments rates of Rb absorption were determined under conditions of constant Rb concentrations. After an absorption period of 30 minutes, the roots were alternately washed several times for about 10 minutes with 50 ml of $2 \times 10^{-3}$ M KCl solution or water. The radioactivity in the roots was determined afterashing at 500°. Absorption rates were calculated from these values. A few results with mung beans (Phaseolus aureus) are also reported. The method followed is similar to that for barley roots except 50 mung bean root sections of 3 cm length were used.

Results

Some Rb absorption rates for barley roots under constant Rb concentrations are shown in figure 1. Each point represents the average of 2 determinations. Data for mung bean roots obtained in another experiment are presented here for comparison. Mung bean results are based on number of roots of 1 length. When plotted on log scales, the results are reasonably linear over a considerable Rb concentration range for both plants. The values of $n$ are the slopes of the lines. The effect of temperature on the parameter $n$ in equation I for barley roots was also studied but it is not reported here. It will suffice to mention that the slope increased with decreasing temperatures, but for a given temperature it also remained constant over the same range in concentration.

The value of $n$ in equation I for barley is 0.17. By measuring concentration changes during absorption, the constancy of $n$ can be ascertained more accurately by use of equation II (10). It is to be noted that the power of $[C]$ in equation II is $1-n$, or 0.83, for Rb absorption by barley roots. Data illustrating the time course of external concentration during uptake are plotted in figures 2 to 4 over the concentrations from $3 \times 10^{-3}$ down to $10^{-6}$ M. These results
Fig. 3. (left). Time-course of disappearance of Rb during absorption by excised barley roots under conditions of changing Rb concentrations. Initial Rb concentration was 100 μmoles/liter, 0.3 g roots in 30 ml.

Fig. 4 (right). Time-course of disappearance of Rb during absorption by excised barley roots under conditions of changing Rb concentrations. Initial Rb concentration was 10 μmoles/liter, 0.3 g root in 30 ml.

Fig. 5. Effect of temperature on the disappearance of Rb during absorption by excised barley roots under conditions of changing Rb concentrations. Initial Rb concentration was 10⁻⁶ M. Root weight to volume was as follows: 22°, 0.2 g to 40 ml; 11°, 0.4 g to 40 ml; 6°, 0.8 g to 40 ml.

Fig. 6. Effect of high RbCl concentrations on Rb uptake by Trebi barley roots in the absence and presence of Ca in 10⁻³ M Tris, pH 8.4. At each concentration the Rb: Ca molar ratio was 2:1 where Ca was added. Both axes on logarithmic scale.

indicate that \( n \) is constant over this concentration range.

Below concentrations of 10⁻⁶ M, Rb absorption by barley roots deviates from equation II. One can see this in figure 5 where the process appears to become first order at 22°. This first order relationship holds below 5 × 10⁻⁷ M when the temperature is lowered to 11°, but is not valid above 5 × 10⁻⁸ M at 6°.

Deviations from equation I can also occur at concentrations above 3 × 10⁻³ M when another variety of barley is used (fig 6). The presence of Ca in the absorption medium, however, prevents the deviation.

Discussion

An original purpose for using equation I and its integrated form, equation II, was to determine the apparent reaction order for uptake of an ion, Rb⁺, as a test for the applicability of the Michaelis-Menten equation to salt uptake. The principles of enzyme kinetics require the rate of uptake to change from zero order, at high concentrations, to first order with respect to substrate concentration, at low concentrations (7). Van’t Hoff (10) suggested that the order of a reaction apparently involving only one reactant is given by the exponent \( n \) in equation I. Figure 5 showed that \( n \) becomes one below 10⁻⁶ M at 22° as expected from the Michaelis-Menten equation. However, the value of \( n \) does not approach one at 6° even at 5 × 10⁻⁸ M. This suggests that this limiting value of \( n = 1 \) with decrease in concentration could be due
to the diffusion of ions from the medium to absorption sites becoming the rate-limiting process.

Plots of a form of the Michaelis-Menten equation 
\( v = -K_m v/C + V_{max} \) (6) for the results given in figure 1 are presented in figure 7. Such curvilinear lines appear typical for roots and have been interpreted as implying 2 processes or sites of uptake (5). This interpretation could be valid only if the 2 processes have parameters of some unique relationship to each other such that their combined rates result in a constant apparent order of reaction over a wide range in concentrations. Such an occurrence would appear to be unlikely. Moreover, calculations using equation 1 show that any one process which has a constant apparent order of reaction (except 0 and 1) over a wide range in concentrations manifests a curvilinear relationship on the Hofstee plot (figure 7). Consequently, caution should be exercised when interpreting a curve on the Hofstee plot as evidence for 2 sites.

Other kinds of kinetic data have been interpreted as 2 processes or sites. Epstein (2) has recently reviewed such data. The results presented in this paper do not apply to them; however, they will be considered in future publications.

Recently, Epstein et al. (4) have published data which they have interpreted as strong proof for 2 sites in cation uptake. They found that at low salt concentrations the absorption rate of K or Rb is independent of the nature of the accompanying anion, but at higher salt concentrations the rate is dependent on the nature of the accompanying anion—a higher rate with Cl⁻ than with the SO₄²⁻. They concluded from these results that K and Rb are absorbed through 2 sites when they are with Cl⁻ and only one site when with SO₄²⁻. On the other hand, Jackson and Adams (8) have presented contradictory data which show that the absorption rates for these alkali ions are independent of the form of the accompanying anion even at high concentrations. The reason for these 2 divergent results lies probably in the manner in which each group conducted its experiment. Epstein et al. (4) added some Ca \( (5 \times 10^{-4} M) \) to the absorption medium, whereas Jackson and Adams did not. Consequently, at high salt concentrations the barley roots used by the latter investigators behaved in the same manner as the minus-Ca roots shown in figure 6. Epstein et al. (4) apparently added sufficient Ca to prevent such effects when the anion was SO₄²⁻ but probably not enough when the anion was Cl⁻. I have found that under similar conditions a Rb: Ca molar ratio of 5:1 is necessary to prevent such abnormal increases in the rate of Rb uptake at high RbCl concentrations. Above \( 5 \times 10^{-4} M \) Rb the amount of Ca employed by Epstein et al. (4) is most likely inadequate to prevent such increases by the Cl⁻ ion. The SO₄²⁻ ion appears to be less effective in this respect than the Cl⁻ ion.

Epstein et al. (4) have also showed that Na is more inhibitory to K absorption at higher K concentrations than at lower concentrations. They cite these results as additional evidence for 2 sites. However, since the relative affinity of K ions for absorption sites in barley roots appears to be greater than that of Na ions, one would expect such results even with one site from known principles of base exchange. In addition, at higher salt concentrations an increased accessibility to absorption sites could occur, resulting in the larger, hydrated Na ion competing more effectively against the smaller K ion. Consequently, these kinds of results should not be taken as conclusive evidence for 2 sites.

Although there might be several reasons why the apparent order for Rb absorption is fairly constant and of a fractional value, a likely explanation could be found in the integrated Michaelis-Menten equation (7):

\[
k = (1/t) \left[ 2.3 K_m \log(x_o/x) + (x_o - x) \right]
\]

where \( x_o \) represents the initial substrate concentration and \( x \) the concentration at any time, \( t \). The relative magnitude of the 2 terms in the right side of the equation will determine the order of the reaction. The reaction will be first order when the concentration of substrate is low or \( K_m \) large, and will be or approach zero order when the converse conditions exist. In other words, the attractive forces between Rb⁺ and its absorption site and the build up of concentration near the site, especially at low concentrations (1), would tend to make and maintain the apparent order of the reaction much less than one. If this explanation is right, correction for these effects might be necessary when using the Michaelis-Menten equation over a wide concentration range. It is of interest to point out here that according to Moelwyn-Hughes (11) surface reactions are characterized by a fractional order, and the rate equation for such reactions is similar to the equation for a Freundlich adsorption isotherm.
At high Rb concentrations, departure from the linear form of equation Ia is observed under certain conditions (fig 6). This can be due to the high salt concentrations bringing about changes in the membrane resulting in an increase in absorption sites (or pores) and/or their becoming more accessible (or porous). Salt absorption under such conditions could become one of mass flow, especially if ions move through charged pores. Although Atlas barley roots do not show a change in the order up to $5 \times 10^{-3} \text{M}$ RbCl in an alkaline medium, Trebi barley roots show an increase in the order above $3 \times 10^{-3} \text{M}$ RbCl in an alkaline medium and from $6 \times 10^{-3} \text{M}$ RbCl in a slightly acid medium (pH 5.7). This increase can be prevented by Ca. In the first medium a Rb: Ca molar ratio of 2:1 is necessary to prevent the increase, but in the second medium the ratio is 5:1. In other words, Rb absorption rates in the presence of Ca fall along the dashed line in figure 6 as expected, and the amount of Ca required decreases with decreasing pH. These results support the well known observation of the essentiality of Ca for membrane integrity. Perhaps, there might be some relationship between this phenomenon and sensitivity of some plants to saline conditions.

At concentrations where $n$ is reasonably constant, the constancy of its value might be utilized to determine absorption rates for such ions as NH$_4^+$, Mg$^{++}$, and possibly NO$_3^-$ for constant concentrations. Such data for these ions are scarce because of the lack of suitable radioisotopes. By following the concentration changes during absorption and applying equations 2 and 1, absorption rates at constant concentrations can be calculated.

If the explanation utilizing the integrated Michaelis-Menten equation to explain the constant and fractional value of $n$ is correct, this value might be used as a relative measure of affinity between an ion and its site. When interpreted in this manner, the smaller values of $n$ for barley roots than for mung bean roots (fig 1) would suggest that barley roots have a relatively stronger affinity for Rb ions than mung bean roots.

Since the results presented showed that the rate of Rb absorption can be represented by an equation similar to that for the Freundlich adsorption isotherm, it is concluded that this kinetic analysis of Rb absorption by barley roots indicates that adsorption—adsorption near absorption sites and not that on other charged sites—plays an important role in the process of cation absorption. An exchange reaction is probably also involved at absorption sites (9).

**Summary**

Rubidium absorption by excised barley roots (*Hordeum vulgare* L. var. Atlas 46) was studied under conditions of constant and changing Rb concentrations over a wide range in concentrations. Under constant concentration, the rate of absorption in an alkaline medium was found to fit an equation of the form, rate of absorption = constant $x$ concentration, from about $10^{-6}$ to $5 \times 10^{-3}$ M. The integrated form of the equation gave an expression which related the concentration to time during uptake under conditions of changing Rb concentration. The exponent $n$ was interpreted to give an apparent order of the absorption process or the coefficient of the Freundlich adsorption isotherm.

An abnormal increase in the apparent order was shown by Trebi barley roots at high Rb concentrations. This increase can be prevented by Ca.

Graphical methods of analysis are proposed to determine relative affinities of plant roots for cations and absorption rates for ammonium, magnesium, and possibly nitrate ions.

It is concluded that Rb absorption by roots is probably an adsorption-exchange process.

**Literature Cited**