Specific Binding of Rubidium in *Chlorella*

**DAN COHEN**

From the Department of Agriculture, University of Oxford, England. Dr. Cohen's present address is the Department of Botany, The Hebrew University of Jerusalem, Israel.

**ABSTRACT** Specific binding sites for potassium, which may be components of the carriers for active transport for K in *Chlorella*, were characterized by their capacity to bind rubidium. A dense suspension was allowed to take up Rb$^{86}$ from a low concentration of Rb$^{86}$ and a high concentration of ions which saturate non-specific sites. The amount bound was derived from the increase in the external concentration of Rb$^{86}$ following addition of excess potassium. The sites were heterogeneous. The average affinity of Rb and various other ions for the sites was determined by plotting the degree of displacement of Rb$^{86}$ against log molar concentration of the individual ions. Interpolation gave the concentration for 50 per cent displacement of Rb, which is inversely related to affinity. The order of affinity was not changed when the cells were frozen, or boiled either in water or in 70 per cent ethanol. The affinity is maximal for ions with a crystalline radius of 1.3 to 1.5 Å and a high polarizability, and is not related to the hydrated radius or valency. It is suggested that binding groups in a site are rigidly arranged, the irregular space between them being 2.6 to 3.0 Å across, so that affinity is high for ions of this diameter and high polarizability.

**INTRODUCTION**

Specific transport of potassium across cellular membranes and specific activation of some enzymes by potassium are in general both assumed to involve a specific binding of potassium. It has been found that the affinity of the transport system for K may be 100 or more times stronger than for either Na or the divalent ions Mg and Ca; but the chemical and physical factors which determine the specificity are as yet unknown; see review by Ussing (1959). The properties of the carriers supposedly operating in the transport of K have been mainly investigated by measuring the competitive inhibition of the transport of K by other ions. However, valid conclusions may only be drawn from experiments involving inhibitions by a second ion if that ion has no influence on any reaction in the transport process other than that relating to binding. Few ions fulfil this requirement; this is well illustrated by the fact that their stimulation or inhibition of the process is frequently non-
competitive (Epstein, 1956). Information gained from competition experiments is therefore limited and in some cases may be misleading.

Few direct investigations concerned with the chemical nature of the K carriers have been made. Even the mildest of chemical reagents are likely to disturb many different reactions in the cell, with the consequence that any observed effect on transport becomes difficult to interpret. It thus appears that little progress can be made as long as assay of the carriers depends on measuring the rate of transport. It was therefore decided to attempt to measure directly the binding of K by cells, since such measurements would eliminate difficulties of interpretation and would allow a more thorough investigation into both the mechanism of binding and the chemistry of the carriers.

The organism selected for study was Chlorella pyrenoidosa, since its capacity to take up K rapidly suggested that it might contain a relatively high concentration of specific binding sites. Also, since it is a small celled unicellular organism, the diffusion path between the external solution and the binding sites might be assumed to be minimal. As the quantities involved were likely to be very small, the use of isotopes seemed clearly to be essential. Because the isotope K\(^{42}\) has a very short half-life, Rb\(^{86}\) was used as a tracer for K. Rb and K are very similar chemically and often have similar affinities for the carrier of K (cf. Fried and Noggle, 1958).

The principle of the method employed in measuring specific binding was to allow the cells to bind Rb\(^{86}\) under conditions in which the non-specific sites, i.e. the "Donnan free space," were saturated by ions which do not compete for the specific sites. The Rb then occupies the specific sites only, and the bound Rb can be measured after displacement by an excess of K. In this way, small quantities of bound Rb can be estimated even in cells possessing a high internal concentration of Rb or K. The competitive powers of other ions were investigated by adding a range of concentrations of the competing ion along with the Rb\(^{86}\) and measuring the amount of Rb\(^{86}\) subsequently displaced by excess K.

That specific binding of K occurs in yeasts has already been demonstrated by Conway and Duggan (1958), while Fried, Noggle, and Hagen (1958) observed a specific binding of K, Rb, Na, and Sr in barley roots. Over a range of concentrations, the quantity bound was proportional to the rate of uptake and it was assumed that the ions were bound by their carriers.

**MATERIAL AND METHODS**

The strain of *C. pyrenoidosa* used in this investigation was 7-11-05 from the collection of type cultures maintained at the Botany School, Cambridge. This is a thermophilic strain, and was chosen because active accumulation of Rb by such cells can be almost completely arrested by cooling to 0–1°C, and this is advantageous for the accurate
measurements of binding. The cells were grown autotrophically in tall (43 cm) rectangular "perspex" vessels (23 cm × 4.3 cm) at 37°C ± 1 and received a light intensity of 1500 foot-candles from "daylight" fluorescent tubes. The culture medium contained, in mM, urea, 18, MgSO₄, 5, KH₂PO₄, 7.5, K₂HPO₄, 2.5, Ca(NO₃)₂, 0.4, together with, in milligrams element/liter, Fe as Fe-EDTA, 5, Mn, 0.5, B, 0.5, Zn, 0.05, Cu, 0.02, Mo, 0.01, and Co, 0.01. Air mixed with 2 to 4 per cent CO₂ was bubbled in a fine stream through the culture vessels, to keep the cells in suspension and provide CO₂ for photosynthesis. Under these conditions, the growth rate was high and reproducible, the cell mass being doubled every 3 hours. The cells, while still growing exponentially, were harvested at a culture density of 1 to 1.5 gm packed cells/liter.

The culture was continuously subcultured so that the cells might retain their capacity for rapid and uniform growth. Although no precautions were taken to maintain sterile cultures, microscopic examination from time to time showed that no algae other than *Chlorella* were present. Slight bacterial contamination sometimes occurred, particularly in older cultures. The *Chlorella* cells were harvested by centrifugation and it is probable that the bacteria, being much smaller were mostly left in suspension. Rb⁸⁶ was estimated by counting appropriately diluted solutions with a jacket type Geiger tube (Ecko M-6).

**Experimental Procedure**

The cells were first suspended in a standard medium which, in order to saturate the non-specific sites, contained relatively high concentrations of non-competing ions. The medium was made up of MgSO₄, tris-HCl, and tris-phosphate, each at 10 mM, and buffered at pH 6.2-6.7. In most experiments, 0.01 per cent of the non-ionic detergent tween 80 was also included to facilitate complete sedimentation of the cells when the suspensions were centrifuged. Other experiments showed that tween 80 at this concentration did not affect the rate of growth.

The suspensions were next centrifuged and the cells "washed" twice, which involved each time resuspension in a fresh medium by sucking and blowing with a graduated pipette. The medium in a subsequent washing included Rb⁸⁶, usually at 0.05 mM, together with the competing ion under investigation. Within a single experiment, the ionic strength was kept constant by the addition, where necessary, of a non-competing ion, tris for monovalent ions, and Mg for di- and trivalent ions. With certain ions, the standard medium was modified to avoid formation of complexes or precipitates. Where buffers could not be included, the pH of the medium was adjusted to the level of the standard medium and left unbuffered; the cells tended to bring the pH to about 6.

The last washing, in which the medium contained 0.05 mM Rb⁸⁶, was necessary in order to displace any K still found by the sites. Also, during this final washing there was an opportunity for Rb and the competing ion to reach equilibrium with the cells. This was important since when the packed cells were finally resuspended in the medium containing Rb⁸⁶ and the competing ion, it was essential that changes in the concentrations during preparation should be minimal.

Dense "incubation" suspensions (10 to 20 per cent) were then prepared and
accurately made up to volume (usually 5 ml). For equilibrium to be reached, the cells were left in suspension for at least 25 to 30 minutes. After equilibration 1 aliquot of the suspension was removed, centrifuged, and the supernatant solution collected. A second was taken after a 40 to 60 minute interval in order to determine the change in Rb concentration with time. The supernatant solutions from these two samples (A and B of Fig. 1) were transferred to sample tubes containing sufficient dry KCl to give a concentration of 10 mM K, which prevented losses of Rb by adsorption to the glass during further handling, and the concentration of Rb$^{68}$ determined.

One to 4 minutes after the first sample was taken, three 1 ml aliquots of the suspension were placed in small centrifuge tubes containing 0.012 ml of a 1 M solution of KCl and the contents mixed quickly. In these "exchange" tubes the displacement of bound Rb from the cells into the exchange solutions took place, 25 to 30 minutes being allowed for the completion of the process. The first of these tubes, C, was centrifuged 26 to 30 minutes after displacement had started; the second, D, 6 to 8 minutes later, and the third, E, 40 to 55 minutes later. The average concentration of Rb in the supernatant solutions and the average time interval between initiation of displacement and centrifugation were calculated for C and D. The change in Rb concentration in E served as a measure of the slow change in concentration with time in the exchange solution, after the main rapid displacement was complete. The effect of this subsequent slow displacement was allowed for by plotting the concentration against time and extrapolating the line back to the point where displacement started (Y in Fig. 1), thus obtaining a "corrected" value for the Rb concentration in the exchange solution. Similarly, the Rb concentration in the medium of the
incubation suspensions, at the time when this was pipetted into the exchange tubes, was obtained by interpolation on the line joining the points for samples A and B. The difference between this interpolated value, X, and the value extrapolated from the exchange tubes, Y, was taken to be the bound Rb displaced from the cells.

From a consideration of the errors incurred in the various steps of sampling, counting, interpolating, and extrapolating, a figure of 1.5 per cent was estimated to be the coefficient of variation¹ of the corrected concentration, Y, of Rb in the exchange solutions. Similarly, 1 per cent seemed a reasonable figure for the coefficient of variation for the concentration in the incubation solution, X, when the exchange samples were taken. The coefficient of variation of the difference (Y-X) can therefore be expressed by the equation of 1.8 R/(R-1) where R is Y/X (1.8 being = 1.5² + 15). This coefficient of variation becomes very large if the relative increase in concentration in the exchange solution (R-1), is small: it approaches 1.8 when R is large. The assumptions involved in arriving at this equation were tested by calculating the coefficient of variation within nine pairs of duplicate samples in different experiments. The coefficient was found to be 1.68 per cent, which is slightly less than that calculated from the equation, since in these experiments R varied between 1.6 and 2.5. This equation has been used to calculate the order of the standard errors for almost all the experiments and their magnitudes are indicated in the tables and figures.

The errors introduced in estimating the concentrations of the competing ions are more difficult to assess. Five per cent is probably a reasonable estimate of the coefficient of variation within one experiment, and 10 per cent, that between different experiments.

EXPERIMENTAL DESIGN

A wide range of ions was investigated with a view to discovering the factors controlling their affinity for the Rb sites. The crystalline radius, the hydrated radius, the valency, the polarizability, and the electronic structure were considered as possible parameters in determining the affinity. Ions differing in these characteristics were accordingly selected. Their affinity for the sites was measured and related to each characteristic, the degree of correlation indicating the importance of each of these properties in determining the affinity for the sites.

For homogeneous and non-interacting sites, the association constant of Rb itself can be derived from the slope of the line obtained by plotting amount bound against amount bound/concentration (Hofstee, 1960). Further, the association constant of a second ion may be derived from the change in the slope induced by the presence of this second ion. However, when the results obtained in this investigation were plotted according to Hofstee, curves resulted (Fig. 2). The non-linearity of these plots will be discussed later. In certain cases, the dissociation constant of the Rb sites was deduced from the concentration at which half the sites were occupied, after the total capacity had been estimated by extrapolation of the curve.

To achieve a more accurate assessment of affinity, a suggestion of Williams was

¹ Standard error as per cent of the mean.
adopted (1958); this is similar to the approach used by Conway and Duggan (1958). For a given concentration of Rb the amount of Rb displaced from the sites by a range of concentrations of a competing ion is expressed as a percentage of that displaced by 10 mM K, which is regarded as inducing total displacement. Plotting this relative displacement against log molar concentration of the competing ion should produce a symmetrical sigmoid curve if the sites are homogeneous and do not interact (Hofstee, 1960). The concentration causing a 50 per cent displacement is equivalent to the dissociation constant of the complex ion site. The slope of the curve is greatest near this point, which can therefore be determined most accurately by interpolation. A

![Figure 2](image)

**Figure 2.** The relationship between the binding of Rb, by living cells, at different concentrations of Rb, and the ratio binding/concentration. The points from two similar experiments are distinguished by filled and open circles. The maximal degree of binding and the concentration for 50 per cent saturation of the sites are indicated by arrows for both curves. (NaCl replaced tris-HCl in the standard medium with a pH of 5.8; experiment conducted at 0-1°C.)

curve of a different shape is obtained if the sites are heterogeneous or interact. Such a curve may be the resultant of several curves reflecting the heterogeneity of the sites. The concentration for 50 per cent displacement is then the average effect of the different dissociation constants and the relative capacities of the various components of the sites.

The degree of displacement can alternately be expressed as log \( Q \), where \( Q \) is the ratio of sites occupied by a competing ion to those not so occupied. In practice, \( Q \) was taken as the ratio of the percentage displacement by a competing ion over the difference between this percentage and 100 per cent. Thus, \( Q = 1 \) and \( \log Q = 0 \) when the level of displacement is 50 per cent. The plot of \( \log Q \) against log molar concentration of a competing ion should form a straight line with a slope of 1 if one competing ion displaces one Rb ion from homogeneous and non-interacting sites. A deviation from linearity indicates that the sites are heterogeneous. The deviation from 1 of the slope at \( \log Q = 0 \) is a measure of both the heterogeneity and the interaction of the sites, and of the average number of Rb ions displaced by each competing ion (see also Edsall and Wyman, 1958).
Except for $H^+$, the level of displacement by the competing ions was plotted as log $Q$ against log molar concentration. The concentration at log $Q = 0$ was obtained by interpolation, and the slope of log $Q$ at this point measured.

**EXPERIMENTAL RESULTS**

(a) *Experiments with Living Cells*

The affinity of the sites for Na, K, Rb, Cs, and Ba was determined by the standard method already given. These ions were selected in an attempt to assess the relative influence of both crystalline as compared with hydrated radius, and of valency. For the ions of the alkali metals, the crystalline radius increases regularly from Na to Cs, while the hydrated radii of K, Rb, and Cs are almost identical, and only that of Na is larger. The crystalline radius of Ba is similar to that of K, while its hydrated radius is much larger. Results for Ba, a divalent ion, could indicate the importance of valency in the determination of affinity. The degree of displacement of labeled Rb from the sites...
by different concentrations of these ions is given in Fig. 3, with relevant experimental details included in the legend.

The results show that the affinities of the ions for the sites fall in the following order: Rb = Ba > K > Cs ≫ Na. The full significance of these findings will be discussed later. For the present it may be noted that the affinity of K is not very different from that of Rb, and that therefore, the use of Rb as a "tracer" for K is legitimate. The 100-fold difference between the affinities of K and Na for the sites indicates that the binding is specific.

Measurement of competition by H ions gives an indication both of the pKa of the site and of the most suitable pH to which the standard medium should be adjusted. The binding of Rb was therefore determined over a range of pH values. Maximum binding was found at pH 6.2 in one experiment and at pH 6.8 in a second. For comparative purposes, the displacement has been expressed as a percentage of the maximum in each experiment and the results are given in Fig. 4. It is seen that there is a good agreement between the two sets of data. Seventy-five per cent of the bound Rb was displaced between pH 6 and 4, and of this fraction, half was displaced at pH 5.2–5.3, which may be taken as the apparent pKa of that fraction of the sites. There
was little further displacement when the pH was lowered to 3.1, suggesting that the pKa of the remaining 25 per cent of the sites is less than 2.2. In all later experiments, the pH of the standard medium was adjusted to 6.2–6.7, since maximum binding was obtained within this range.

To test whether the binding sites are, in fact, the carriers concerned in active uptake, the affinity of Rb for the carriers was determined and compared with that for the binding sites. Suspensions of cells (5 per cent v/v) were shaken in different concentrations of Rb in the standard medium (pH 6.6) at room temperature (21°C) under diffuse light. The rates of uptake were derived from the falls in concentration of Rb in the external solutions. In Fig. 5, this rate is plotted against rate/concentration, and the non-linear relationship indicates that the uptake sites are heterogeneous. However, the curve may be tentatively resolved into two straight lines. The shape of the curve and the affinity of Rb for the carriers are thus similar to those obtained for the binding sites (Fig. 2).

(b) Experiments with Killed Cells

The investigation into the affinity of the sites for a wider range of other ions was limited by the fact that some ions cause injury to living cells. Leakage of internal K commonly follows injury and may seriously interfere in the determination of bound Rb in cells. At this time, other research in the Department on the uptake of 2,3,5-triiodobenzoic acid was demonstrating that some of the components concerned in the uptake process were not destroyed when the tissues were placed in liquid air and then thawed (see Blackman and

---

**Figure 5.** The relationship between the rate of uptake of Rb by live cells and the ratio rate/concentration. The standard medium (pH 6.6) was employed at room temperature (21°C). The curve was resolved into two lines; the Michaelis constants corresponding to their slopes are indicated.
Sargent, 1959). It seemed therefore that there might be a way of killing *Chlorella* cells without destroying the binding sites, thereby avoiding the injurious effects of toxic ions during the determination of binding.

The remarkable fact was established that the binding sites remain stable even though the cells are killed by freezing and thawing, by boiling in water, or in 70 per cent ethanol or acetone.

Subsequently, it proved to be convenient to use killed cells not only in competition experiments involving toxic ions but also for other ions, since in killed cells interference from metabolic and permeability factors is eliminated. The standard method employed for killing the cells by heating was to immerse the vessel containing the cell suspension in a boiling water bath for 30 minutes;
a temperature of 95°C was usually reached in 5 to 8 minutes. Alternately, extraction with boiling 70 per cent ethanol was continued until almost all the pigments were removed; three changes of the solvent were usually required. To kill the cells by freezing, the vessel was immersed in liquid air or in a mixture of solid CO₂ and ethanol until the contents were frozen. The cells were then thawed and the operation repeated two to three times. After killing, the cells were washed and the binding of Rb measured in the usual way, either at room temperature (18–23°C), or in an ice bath.

First, the affinity of the sites in cells killed by heating for Na, K, Rb, and Ba was reexamined and the results compared with those already found for the same ions in living cells. Displacement of labeled Rb from the sites by different concentrations of these ions and of NH₄ is shown in Fig. 6. Comparing these results with those shown in Fig. 3, it is clear that the affinities of the sites for these ions are very similar in both living and killed cells. It was therefore assumed that affinities determined for other ions in killed cells only, can be taken as indicative of their affinities for the sites in their natural state.

Other ions were selected for investigation according to the criteria already outlined. Manganese was taken as representative of the ions of the transition metals, which all have strong coordinating powers. However, the crystalline radius of Mn is smaller, and its hydrated radius larger than those of Rb or K. Thus, if the affinity for the sites is determined by either of the radii, Mn should have a low affinity. The results of two estimations are given in Fig. 7. The agreement between the two experiments was not good and the degree of displacement was low even for the highest concentration of Mn: extrapolation to the point where log Q = 0 is not therefore accurate. In one of these experiments, competition by magnesium was also examined but no displacement was detected even at a concentration of 10 mM. Thus, both Mn and Mg behaved as if the radius, either crystalline or hydrated, was more important in determining the affinity than was either valency or strong coordinating powers.

Trivalent lanthanum and divalent strontium have similar crystalline radii, intermediate between those of Na and K. Their hydrated radii are different, however, both being larger than that of Na. The effects of competition by these ions are also given in Fig. 7; seemingly the affinity of both ions is largely dependent on their crystalline radii.

Both crystalline and hydrated radii of monovalent thallium are similar to those of Rb. Tl⁺ is heavier and more polarizable, and its affinity constant is therefore of considerable interest. The results for the competition by Tl are also given in Fig. 7. Comparison of Figs. 7 and 6 reveals that the affinity for Tl is about four times that of Rb. It can therefore be concluded that polarizability is important in determining affinity.

Uranyl (UO₂⁺⁺) is a dumb-bell-shaped ion, similar to K in cross-section,
but with a considerably greater long axis. If affinity is determined by the
radius in the three coordinates of space, then the affinity of uranyl should be
low. Uranyl also has a high affinity for polyphosphate groups which makes
the determination of its affinity for the sites of some significance. In the

Figure 7. The displacement of Rb by a range of concentrations of Mn, La, Sr, and
Tl⁺. The cells were killed by boiling for Mn and La, by extraction with ethanol for Sr,
and by freezing and thawing for Tl. (For Mn, the medium contained 10 mM MgCl₂
and 20 mM tetramethyl ammonium nitrate at pH 6.2–6.6. For La, the medium con-
sisted of 10 mM MgCl₂ and 20 mM tris-HCl at pH 6.5. For Sr, the medium was made
up of 10 mM each of MgCl₂, tris-HCl, and imidazole succinate at pH 6.5. For Tl, the
standard medium included 1 mM NaCl at pH 6.6. For all experiments, the temperature
was 19–20°C.)

assessment, cells killed by heating were used, and the determination was
carried out at room temperature in the standard medium at pH 6.3, save
that the tris-phosphate was replaced by 1 mM succinate-imidazole buffer.
The cells were divided into six portions, and each portion was washed three
times in the same concentration of uranyl as that used during the measurement
of binding. At the highest concentration which could be attained at the pH
of the medium, \( i.e. \) 0.1 mM, no displacement was apparent. The affinity of uranyl for the sites is thus low, and any polyphosphate groups present in the sites must be inaccessible.

The results for all the competition experiments, save those for H ions, are summarized in Table I, where for both living and killed cells, the concentrations required for 50 per cent displacement and the mean slope of \( \log Q \) are presented for each ion, together with the standard errors where they are appropriate.

The temperature coefficient of the affinity for Rb was found to be small.

\[ \text{Table I} \]

<table>
<thead>
<tr>
<th>Cation</th>
<th>Type of cell material</th>
<th>Concentration for 50% displacement</th>
<th>Mean ( m_a )</th>
<th>Slope ( \log Q )</th>
<th>Mean slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>Living</td>
<td>29.0</td>
<td>27.5±1.5</td>
<td>0.48</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Na</td>
<td>Heat-killed</td>
<td>26.0</td>
<td>26.0</td>
<td>0.56</td>
<td>0.96±0.09</td>
</tr>
<tr>
<td>K</td>
<td>Living</td>
<td>0.27</td>
<td>0.29±0.019</td>
<td>1.03</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>K</td>
<td>Heat-killed</td>
<td>0.31</td>
<td>0.31</td>
<td>0.87</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Rb</td>
<td>Living</td>
<td>0.12</td>
<td>0.12</td>
<td>0.81</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Rb</td>
<td>Heat-killed</td>
<td>0.14</td>
<td>0.14</td>
<td>0.81</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Rb</td>
<td>Extracted in ethanol (30°C)</td>
<td>0.153</td>
<td>0.123±0.003</td>
<td>1.02</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>Rb</td>
<td>Extracted in ethanol (18°C)</td>
<td>0.11</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs</td>
<td>Living</td>
<td>1.16</td>
<td>1.21±0.05</td>
<td>0.63</td>
<td>0.70±0.07</td>
</tr>
<tr>
<td>Cs</td>
<td>Heat-killed</td>
<td>1.26</td>
<td>1.26</td>
<td>0.77</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>NH₄</td>
<td>Heat-killed</td>
<td>0.61</td>
<td>0.61</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Tl⁺</td>
<td>Killed by freezing</td>
<td>0.03</td>
<td>0.03</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>Living</td>
<td>0.10</td>
<td>0.10</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>Heat-killed</td>
<td>0.16</td>
<td>0.16</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>Extracted in ethanol</td>
<td>7.95</td>
<td>7.95</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>La</td>
<td>Heat-killed</td>
<td>10.1</td>
<td>10.1</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>Heat-killed</td>
<td>100.0</td>
<td>100.0</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Uranyl</td>
<td>Heat-killed</td>
<td>( \sim &gt;4 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>Heat-killed</td>
<td>( \sim &gt;400 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetramethyl ammonium</td>
<td>Heat-killed</td>
<td>( \sim &gt;800 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The binding of Rb by ethanol-extracted cells was measured in an ice bath (0–1°C) and at room temperature (21°C) and there was no significant difference between the concentrations of Rb required to saturate 50 per cent of the sites at the two temperatures. The change in enthalpy during binding is thus much smaller than the free energy of binding.

Preliminary experiments had shown that displacement of bound Rb by 10 mM K is completed within 25 minutes and it was decided to measure more accurately the rate of displacement. A single dense suspension of heat-killed cells was prepared which contained approximately 0.05 mM Rb. Samples were withdrawn from the suspension on four occasions. After each sampling, 5 aliquots of the suspensions were rapidly transferred to centrifuge tubes containing sufficient 1 M KCl to make up a final concentration of 10 mM K. The contents were mixed quickly and the tubes shaken continuously during the experiment. The tubes were centrifuged after intervals of 1.5 to 196 minutes to determine the concentration of Rb in the solutions. It was found that after about 30 minutes there was no further increase in concentration and all bound Rb in the cells was assumed to have been displaced by that time. This increase in concentration was identical for the four series of tubes. The log of the remaining bound Rb in the cells, as a percentage of the total, was then plotted against the time required for displacement. The results for the four series of tubes were in good agreement and on the assumption that the displacement is a first order process, the curve formed by the points was resolved into two lines with widely differing slopes. From the slopes and intercepts of the lines, it was deduced that about 90 per cent of the bound Rb was displaced at a high rate constant of 0.55 mol bound Rb/mol bound Rb/minute, and the other 10 per cent at a rate constant of 0.025/minute. It is therefore apparent that the 25 to 30 minutes allowed for equilibration of cells and solutions during the measurements of binding throughout this investigation was sufficient.

**DISCUSSION**

On the basis of the results reported, it can be concluded that there are sites in *Chlorella* which bind Rb with a high affinity. The affinity of these sites for an ion is seemingly dependent upon the crystalline radius but is clearly not related to the hydrated radius or the valency. These relationships are well brought out in Fig. 8, where the concentrations required for 50 per cent displacement of labeled Rb are plotted against the crystalline and hydrated radii. It is evident that the affinity is low for small ions, but increases regularly with the radius to a maximum near 1.4 Å. However, between 1.4 and 1.69 Å, the affinity decreases as the radius increases to that of Cs, and becomes very low for the large tetramethyl ammonium ion. Apparent exceptions to this
relationship are ammonium, of which the affinity is too low, and Tl of which it is too high.

A hypothetical structure of the sites which would account for the affinities for all the ions is as follows. Binding groups within the site are arranged in a rigid pattern, the space between them being 2.6 to 3.0 A across. The tendency for an ion to be bound within this space would be maximal for those ions which can make closest contact with all binding groups. Because of these spatial restrictions, the affinity of the sites will be greatest for ions with a radius of 1.3 to 1.5 A, and will decrease for ions with a smaller or larger radius. As shown in Fig. 8, it is the crystalline radius that determines the order of affinity, suggesting that the ions are unhydrated when bound. The change
in free energy during binding of hydrated Rb is thus the sum of the energy of binding of unhydrated Rb by the unhydrated sites, the energy of dehydration of both Rb and the site, and the energy of hydration of the complex. This suggestion would fit in with the view that in general the formation of complexes by an aqueous ion is an equilibrium between binding and hydration (Bjerrum, 1941).

Binding of ions according to this model is analogous to the binding of ions by zeolites and phosphomolybdates, where the dimensions of the crystal lattice restrict binding to ions of a limited size (Barrer and Meier, 1958; Smit, Jacobs, and Robb, 1959). Steric factors are also important when the size of an ion has a marked effect on the stability of its complexes (Schwarzenbach, 1954). Similarly, uptake of iodine by thyroid tissue of rats is inhibited by an ion of the same size, but is unaffected by larger or smaller ions (Anbar, Guttman, and Lewitus, 1959).

It is assumed that the shape of the space is not regular. To ensure the closest contact with the binding groups, a bound ion has to be deformed so that the affinity will be greatest for ions that are most easily deformed. For monoatomic ions, the polarizability can be taken as a measure of the ease with which an ion can be deformed. The polarizability of some ions is given in Table II. It seems that the greater polarizability of larger ions increases their affinity for the sites. Cs, which is too large for maximal affinity, can easily be deformed, thus coming in contact with more binding groups. Ions that are smaller than optimal are also less polarizable. Even if these ions are only slightly too small, they cannot make contact with all the binding groups. There is thus a greater difference between the affinities of K and Sr than between those of Rb and Cs, although the difference between their radii is almost the same. The greater polarizability of Tl can account for its high affinity relative to that of Rb, which has a similar radius. The polarizability of ammonium is almost entirely due to the displacement of electrons in the N—H bonds (Syrkin and Dyatkina, 1950). Since the length and angles of the latter are relatively

<table>
<thead>
<tr>
<th>Table II</th>
</tr>
</thead>
<tbody>
<tr>
<td>THE POLARIZABILITY OF GASEOUS IONS IN A^4 (PAULING, 1927)</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Monovalent</strong></td>
</tr>
<tr>
<td>Na</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>Rb</td>
</tr>
<tr>
<td>Cs</td>
</tr>
<tr>
<td>NH₄</td>
</tr>
<tr>
<td>Tl</td>
</tr>
</tbody>
</table>
rigidly fixed, the ion is not easily deformed, and this may explain why the affinity of ammonium is lower.

Binding may be mainly electrostatic; indeed it is difficult to envisage a covalent binding of the alkali metal cations. The high rate of displacement of Rb by K, characteristic of ion exchange reactions, supports this suggestion. So does the fact that the uncharged water molecules, which are similar in size and polarizability to Rb ions, do not interfere with binding. An electrostatic mode of binding is also indicated by the negligible change in enthalpy, ΔH, during binding. See Klotz and Fies (1951).

![Figure 9](image-url)

**Figure 9.** The relationship between the slope of log Q and log concentration for 50 per cent displacement. The symbols are: circles for monovalent, triangles for divalent, and squares for trivalent ions.

When results for all the competing ions are compared (Fig. 9), it appears that the slope of the curve relating the degree of displacement of Rb, log Q, to the log molar concentration of a competing ion is more than 0.9 for monovalent ions with a high affinity for the sites. The slopes decrease for both monovalent ions with a low affinity and for polyvalent ions.

According to Edsall and Wyman (1958) a low slope of log Q/log molar concentration can be accounted for (a) by binding by heterogeneous sites; or (b) by a negative interaction between binding by adjacent sites. For Rb and other monovalent ions with a high affinity, slopes of log Q are greater than 0.9, suggesting that the sites are heterogeneous only to a small extent. Thus slopes of log Q lower than 0.9, found for other ions, would be due to a negative interaction between ions bound at adjacent sites. An electrostatic
repulsion between such ions may cause this interaction. Such a repulsion would increase with the valency of the ions, thereby reducing the slope of log Q. The decrease of slope with affinity for ions of the same valency is perhaps caused by a more effective neutralization or screening of the ionic charge of a firmly bound ion.

Electrostatic repulsion between bound ions may account for the similar affinities for the sites of ions with similar radii and different valencies. Increased attraction between a polyvalent ion and the negative charge in the site would be counteracted by increased repulsion between such ions on adjacent sites. In an analogous manner, phosphomolybdates and some zeolites bind monovalent ions more firmly than they do divalent ions of the same size. Electrostatic strains in the crystal lattice caused by the binding of divalent ions by monovalent sites, reduce the energy of binding these ions (Barrer and Meier, 1958).

The binding sites have several characteristics in common with the carriers supposedly operating in active uptake of Rb and K. The curve relating rate of uptake of Rb by Chlorella to rate/concentration was similar to that for binding of Rb (compare Figs. 2 and 5). Similar curves have also been obtained for the uptake of Rb and K by barley roots (Fried and Noggle, 1958). As a result of their study of uptake over a range of pH's, these authors concluded that the pKα of the carriers is about 5. A similar pKα for the carrier in the case of Chlorella was indicated by preliminary experiments in the present investigations. While uptake of Rb was rapid above pH 5, it was completely stopped when the pH reached 4.1.

The fact that all the sites are apparently equally available in both living and killed cells, coupled with the high rate of displacement in both types of cells, indicates that the sites are located external to any permeability barrier. The sites may therefore be located in the membrane constituting the permeability barrier, where they may act as part of the transport system. The specificity of binding would thus determine the specificity of transport.

Finally it should be pointed out that if all the sites are in fact involved in transport, their rate of turnover, as calculated from the figures for maximal rate of active uptake and for binding capacity, is about 5 × 10⁻⁴/sec. which is very low in comparison with similarly derived figures for erythrocytes (Glynn, 1957). This divergency suggests that not all the sites are part of the carrier system.

The work was carried out while the author held the Reginald Graham Scholarship at Pembroke College, Oxford, for which I am grateful to the College and to the Friends of the Hebrew University in Great Britain. I am greatly indebted to my supervisor, Professor G. E. Blackman, F.R.S., for his continuous help and encouragement. I wish also to thank Miss M. A. Pratt, Dr. L. C. Reinhold, and Dr. J. Dainty for their help in preparing the manuscript, and Dr. B. Z. Ginsburg and Dr. R. J. P. Williams for stimulating discussions.
This paper includes part of the material of a Doctor of Philosophy thesis submitted to the University of Oxford.

Received for publication, December 18, 1961.

REFERENCES


BJERRUM, J., Metal Ammine Formation in Aqueous Solutions, Copenhagen, Haase and Son, 1941, 77–80.


WILLIAMS, R. J. P., private communication, 1958.