# DEMONSTRATION OF SATURABILITY AND COMPETITION IN ION TRANSPORT INTO A MEMBRANELESS PROTEIN-WATER SYSTEM

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SUMMARY

The initial rate of uptake of labeled $Rb^+$ ion into a membraneless actomyosin gel system shows "saturability" and "competition," as has been demonstrated in the rate of entry of ions, amino acids, and sugars into a variety of living cells. The present study shows that the demonstration of "saturability" and "competition" does not constitute evidence that the rate of entry is surface or membrane-limited.

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In 1952, Epstein and Hagen demonstrated that the rate of entry of $Rb^+$ ion into barley roots ($V_i$) could be described by an equation of the following form:[1]

$$V_i = \frac{V_i^{max} [p_i]_{ex} \tilde{K}_i}{1 + [p_i]_{ex} \tilde{K}_i + [p_j]_{ex} \tilde{K}_j} \qquad (1)$$

where $[p_i]_{ex}$, represents, in this case, the external $Rb^+$-ion concentration; $[p_j]_{ex}$ is the concentration of a competing solute of the same type (e.g., $K^+$ ion) also present in the external medium; and $\tilde{K}_i$ and $\tilde{K}_j$ are adsorption constants of the $i^{th}$ and $j^{th}$ species, respectively, (in units of $M^{-1}$) on certain postulated inward moving "carriers" in the cell membrane.

Equation 1 shows that the rate of entry does not increase steadily at high external $Rb^+$-ion concentrations but approaches a limiting value, $V^{max}$ (Saturability). Equation 1 also shows that $V_i$ is dependent on the concentration of the competing species, $[p_j]_{ex}$ (Competition).

Epstein and Hagen's finding has been confirmed in a variety of living cells.[2-10] However, their interpretation on the basis of the fundamental postulates of the membrane theory,

although widely accepted, is not the only one that has been offered. It has been shown, on the basis of the association-induction hypothesis, that conformity to Equation 1 may be anticipated in the rate of entry of solute into any nonliving or living systems which bear appropriate fixed sites that can adsorb the solute being investigated.[3,4] In support of this theory it has been shown that similar saturability and competition have been observed in the rates of entry of ions into a variety of nonliving systems as well. These include cation exchange resin sheets, and sheep's wool.[3,4,9]

So far, the theoretical models examined have been developed with the implicit assumption that the cell surface is the rate-limiting step that determines the rate of solute entry into the cells. The question arises whether conformity of solute entry to an equation having the form of Equation 1 can be considered as evidence that the rate-limiting step is, in fact, the cell surface. The present study represents an experimental attempt to answer this question.

## MATERIALS AND METHODS

### *Materials*

All chemicals used were of cp grade. Rubidium chloride (99.99%) was obtained from Penn Rare Metals Inc., Revere, Pa., and $Rb^{86}$ from Union Carbide Corp., Oak Ridge National Laboratories, Oak Ridge, Tenn.

### *Preparation of Actomyosin Gel*

Actomyosin (myosin B) was prepared by a modification of the method of Szent-Györgyi.[11] Minced rabbit muscle was extracted with 3 volumes of a modified Weber-Edsall solution (0.75 M KCl, 0.01 M $Na_2CO_3$, 0.04 M $NaHCO_3$) and squeezed through fine gauze. The viscous liquid obtained was diluted with one-tenth of its volume of the same solution and centrifuged at O°C, first at 8000 x g for 30 minutes and then at 20,000 x g for 30 minutes, to remove bits of connective tissue. The resulting viscous fluid was diluted with water to a final $K^+$-ion concentration of 0.1 M to precipitate the gel. After centrifugation at 13,000 x g for 30 minutes, the supernatant was decanted and the gel was dissolved by adding 1.2 M KCl to a final $K^+$-ion concentration of 0.6 M. The gel was precipitated by dilution to a $K^+$-ion concentration of 0.05 M, again dissolved in a 0.6 M $K^+$ ion, and finally precipitated by dilution with distilled water to a final $K^+$-ion concentration of 0.007-0.008 M.

### *Rate of Entry Studies*

1.0 ± 0.05 gram portions of the final gel were distributed to a series of 50 ml Nalgene centrifuge tubes each containing 10 ml of a 5.0 mM KCl solution. For this process plastic 20 ml syringes (Becton and Dickinson) were used. The exact amount of gel added to each

tube was determined by weighing the syringe before and after each delivery. (The adsorption of ions onto glass at these low ionic concentrations renders glass tubes unusable in these experiments.) The mixture in the centrifuge tubes sat at 4°C for 16 hours, after which the gel was carefully separated from the supernatant fluid after centrifugation at 23,000 x g for 30 minutes. Any solution adhering to the tube was removed by wiping the inside of the tube with rolled filter paper and cotton swabs. The gel remained solidly packed and the surface layer undisturbed during this and subsequent procedures. The labeled experimental solution used for this study of $Rb^+$-ion entry into the actomyosin gel contained 0.1 $\mu$Ci/ml of $Rb^{86}$, 0.2 mM glyclglycine buffer, 0.1 mM RbOH and concentrations of RbCl varying from 0.1 mM to 1.9 mM. In studies where a competing ion was present, 1 mM of RbCl was replaced by the chloride salt of the competing cation.

From a polypropelene graduated cylinder, 10 ml of the experimental solution were poured carefully down the side of the tube containing the packed gel.

After a specified length of time, the solution was decanted and replaced with 15 ml of the washing solution (distilled, deionized water, 0°C). The washing solution was decanted after 10 seconds and the tube cleaned of any adhering solution. After the weight of the gel was determined, the gel was solubilized by the addition of 1 N NaOH directly to the tube.

The radioactivity of a 2 ml aliquot was assayed on a well-type y-scintillation counting system. Three drops of 6 N HCl were added to 5 ml of the experimental solution to prevent subsequent cation uptake by glass pipettes. Two-ml aliquots of this acidified solution were pipetted into counting tubes for assay of radioactivity. A second portion of the supernatant was used for pH measurement.

## RESULTS AND DISCUSSION

Figure 1 shows two examples of the time course of labeled $Rb^+$-ion uptake by the actomyosin gel.

Within the first minutes the curves are close to being rectilinear. In the experiments to be described, the uptake of labeled $Rb^+$ ion after 15 minutes of incubation was studied.

Figures 2 and 3 show two examples of the study of labeled $Rb^+$-ion entry into the actomyosin gel in the absence (open circle) and presence (half-filled circle) of a competing ion. In these figures, the reciprocal of the initial rate of entry was plotted against the reciprocal of labeled $Rb^+$-ion concentration. Straight lines were obtained whether or not a competing ion was present. The intercepts of these lines converge on the same locus on the ordinate in agreement with the prediction of Equation 1 in the reciprocal form:

$$\frac{1}{V_i} = \frac{1}{\tilde{K}_i V_i^{max}} \left(1 + \tilde{K}_j [p_j]_{ex}\right) \frac{1}{[p_i]_{ex}} + \frac{1}{V_i^{max}} \qquad (2)$$

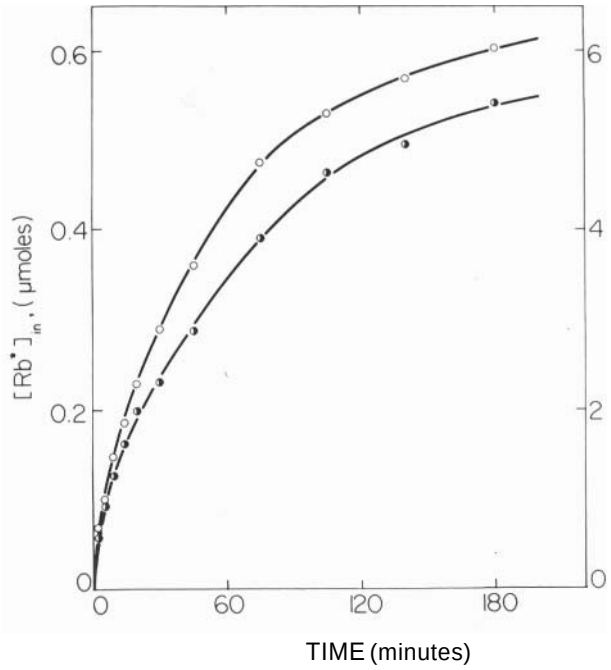


Figure 1. Time course of labeled $Rb^+$ ion entry into actomyosin gel at 25°C. Lower curve (◑) shows uptake at a $Rb^+$-ion concentration of 4.0 mM; upper curve (○) at a concentration of 0.4 mM. Gel "washed" for 10 seconds at 0°C. Each point represents the average of three individual determinations. Protein concentration of gel was 4.96%.

From these and five other sets of data, we obtained an average of $5.57 \times 10^{-6}$ (mole/cm$^2$/15 min) for $V^{max}$ and **102** $(M)^{-1}$ for $\widetilde{K}_i$. As competing ions the average $\widetilde{K}_j$ for $Rb^+$ and $K^+$ ion are, respectively, **147** and **120** $(M)^{-1}$.

These data clearly show that saturability and competition do not imply a surface-limited type of solute transport. Instead, they show that the rate of uptake represents two processes going on simulfaneously: (1) diffusion into the water of the actomyosin gel and (2) adsorption (or exchange adsorption) of $Rb^+$ ion onto protein adsorption sites present on the surface as well as in the bulk of the system. The adsorption of alkali-metal ions on isolated actomyosin has been established by Lewis and Saroff using permaselective electrodes[12] and by Cope using wide-line nuclear magnetic resonance spectroscopy.[13]

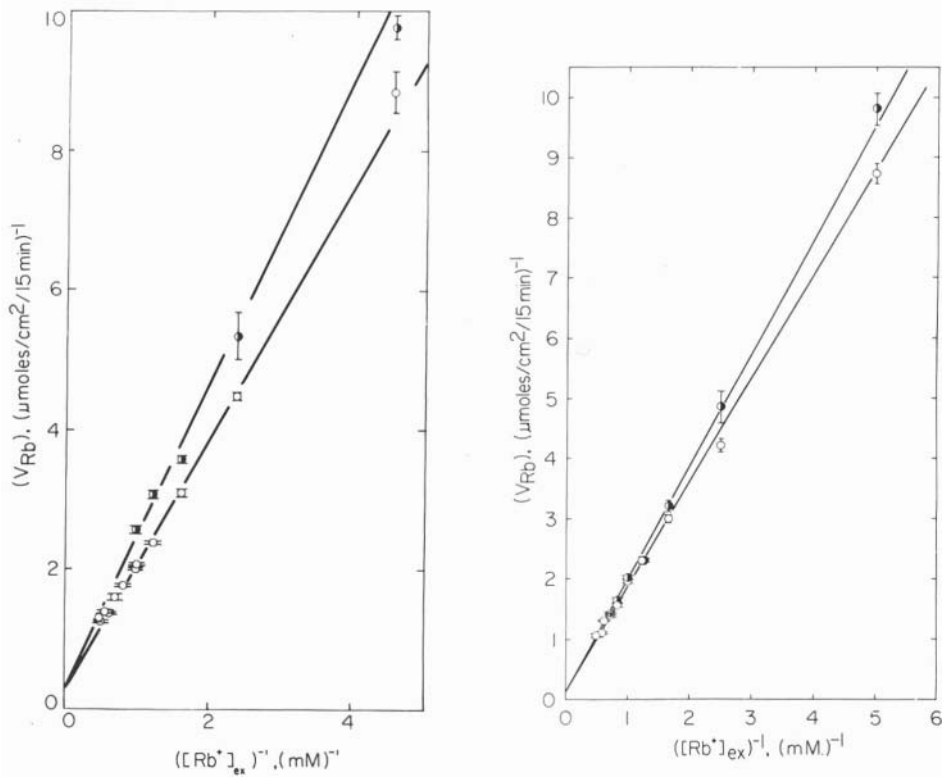


Figure 2. The effect of nonlabeled $Rb^+$ ion on the rate of entry of labeled $Rb^+$ ion into actomyosin gel.
Lower curve (○) represents the rate of $Rb^+$ ion entry with no added competing ion. Upper curve (◑), the entry with 1.0 mM nonlabeled $Rb^+$ ion present. Gel incubated for 15 minutes at 26°C, followed by 10 seconds of "washing" at 0°C. The pH of the final experimental solution and the washing solution was 6.8. The protein concentration of the gel: 4.28%.
Each point represents the average value ± S.E. of four individual determinations.

Figure 3. The effect of $K^+$ ion on the rate of entry of labeled $Rb^+$ ion into actomyosin gel.
Lower curve (○) represents the rate of $Rb^+$ ion entry with no added competing ion. Upper curve (◑), the entry with 1.0 mM $K^+$ ion present. Details same as Figure 2. The protein concentration of gel was 5.2%.

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