

UNSTIRRED MUCUS LAYERS: ION EXCHANGE PROPERTIES AND EFFECT ON ION REGULATION IN *LYMNAEA STAGNALIS*

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(Received 28 September 1981)

SUMMARY

Mucus from the footsole of the freshwater snail *Lymnaea stagnalis* behaves as a weak, negatively charged ion exchanger. Activities and concentrations of Na, K, Ca, and Cl were measured in mucus dialysed to equilibrium against artificial pond water or physiological saline. Observed activity coefficients (activity/concentration) in mucus were compared with those predicted by the Debye-Huckel theory to interpret the effects of electrostatic forces between the polyelectrolyte ions and small ions. The affinity of mucus for small ions decreased in the series, Ca^{2+} , K^+ , Na^+ , Cl^- .

The extent to which mucus can concentrate cations was measured using three different methods: by titrating the fixed acidic groups with K or Ca and by equilibrium dialysis after which the electrical potential difference was either measured directly or was calculated from the Nernst potential for Na. Ion exchange titration indicated a much smaller exchange capacity than did the other two methods.

Kinetics of cation uptake by the snail from dilute media were re-interpreted by considering the enhanced concentrations of cations in the mucus layer. It was shown that the presence of mucus in the unstirred layer adjacent to a transporting epithelium can result in an underestimate of the Michaelis constant (K_m) determined from influx measurements.

INTRODUCTION

The freshwater pulmonate snail, *Lymnaea stagnalis*, takes up ions from the dilute environment in which it lives to compensate for salts lost in urine and faeces and through the integument. Much of this ion uptake is thought to take place across the exposed, mucus-covered integument by active transport against chemical potential gradients. Active uptake has been proposed for Na and Cl (Greenaway, 1970, 1971; de With, Witteveen & van der Woude, 1980); Ca (van der Borgh & van Puymbroeck, 1964); Na, K, Ca, and Cl (Schlichter, 1981).

Greenaway (1970, 1971) showed that Na and Ca uptake exhibits Michaelis-Menten saturation kinetics with half-saturation constants (K_m) of about 0.25 mM. However, because of the unstirred layer of liquid adjacent to the membrane, the solute concentration at uptake sites may not be known. In the unstirred layer the solute concentration

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may decrease as the membrane is approached from the source side and also decrease with distance from the membrane on the sink side (Dainty & House, 1966). Such a profile results when the resistance of the unstirred layer to diffusion is sufficiently large compared with the resistance of the membrane. Measurements of the one-way flux from the source side to the sink side will overestimate the true K_m if the solute concentration at the membrane on the source side is lower than in the bulk solution. Lieb & Stein (1972) and Winne (1973) derived equations showing that the magnitude of the overestimate of K_m will depend on the thickness of the unstirred layer, the diffusion coefficient of the solute in the unstirred layer, and the solute flux through the unstirred layer. The present study concerning an unstirred layer of mucus adjacent to an ion transporting membrane will demonstrate that the opposite bias, an underestimate of the true Michaelis constant, can also be produced.

The entire exposed epidermis of *L. stagnalis* is covered with mucus. Foot mucus glands contain neutral, sialylated, and sialylated-sulphated mucopolysaccharides (glycoproteins) (Zylstra, 1972); therefore, the bulk foot mucus contains polyelectrolyte molecules with fixed negative charges. The electrostatic field surrounding the fixed charges can be expected to increase the number of diffusible cations and reduce the number of diffusible anions as predicted by the classic Donnan equilibrium (Overbeek, 1956). In the present study equilibrium ion concentrations and activities in mucus were measured over a wide range of ion concentrations. The ion exchange properties of this mucus were used to predict the influence of mucus on active ion uptake.

MATERIALS AND METHODS

Mature pond snails, *Lymnaea stagnalis appressa* Say, were collected from a pond in southern Ontario and stored in shallow tanks lined with mud and filled with dechlorinated tap water at 15 ± 1 °C. The snails were fed fresh lettuce daily. Experimental animals were acclimated to artificial pond water (APW) at 21 ± 1 °C for at least two weeks and starved for one day before they were used. Artificial pond water (APW) contained approximately 0.5 mM-NaCl, 0.1 mM-KCl, 0.4 mM-CaCl₂, and 0.3 mM-NaHCO₃. *Lymnaea* physiological saline (Carriker, 1946) contained 34 mM-NaCl, 24 mM-NaHCO₃, 0.7 mM-KH₂PO₄, 3.2 mM-MgCl₂, and 2.7 mM-CaCl₂ and was adjusted with NaOH to pH 7.8.

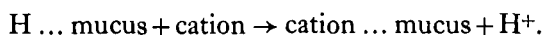
Mucus accumulated at the caudal end of the footsole of snails that were suspended in APW by threads cemented to their shells (Schlichter, 1981). The mucus mass was collected with forceps and stored in closed vials at 4 °C until it was used, at most 6 h after it was collected.

Titration of fixed acidic groups. The ion exchange capacity of whole mucus was measured by titrating the fixed acidic groups. The method is similar to that used by Marshall (1978) for mucus of a teleost fish and is recommended by Helfferich (1962) for ion exchange capacity determination. The general approach is to deionize the mucus sample, convert it to the protonated form, and exchange the protons for K or Ca.

Disodium EDTA was added to 0.5–1.0 ml of mucus from each snail to yield an EDTA concentration of 15 mM. Mucus samples were placed in dialysis sacs (MW cutoff 12 000–14 000) and dialysed for at least 12 h against several changes of distill.

deionized water at 10 ± 1 °C. Dialysis was considered complete when the conductivity of the dialysis water was the same as that of deionized water ($< 2 \mu\text{S}$). Mucus was then converted to the protonated form by adding HCl to bring the pH to 1.5–2.0. The sample was again dialysed against deionized water until the pH and conductivity of the dialysis water were the same as for distilled water. No Na, K or Ca was detected in protonated mucus samples, as measured by atomic absorption or emission spectrophotometry. Each mucus sample was split into three portions. One portion was weighed, dried at 30 °C, and reweighed to determine mucus concentration as g solids/kg protonated mucus. Each of the other two portions was placed in a small plastic vessel and a needle was inserted through which N_2 gas was continuously bubbled to mix the mucus and to drive off CO_2 .

A miniature pH electrode was inserted and the initial, stable pH was recorded. A few microlitres of 0.5 M- CaCl_2 or 3 M-KCl were injected into the chamber and the decrease in pH was monitored until there was no further change in pH. When an excess of Ca or K has been added, the ion exchange reaction can be written as



The ion exchange capacity was calculated as the number of equivalents of H^+ released per gram dry mucus.

Equilibrium dialysis. Samples of deionized, protonated mucus were dialysed for at least 12 h against stirred one-litre volumes of APW or physiological saline or NaCl solutions (1–50 mM) at 10 ± 1 °C. Aliquots of these samples were used for measuring the concentrations and activities of ions and for determining the per cent dry weight.

Ion measurements. Concentrations of Na and K, and of Ca were determined by flame emission and atomic absorption spectrophotometry, respectively. Neutron activation analysis was used to measure Cl concentrations. Chemical activities of Na^+ , K^+ , Ca^{2+} , and Cl^- were measured with ion-selective electrodes calibrated with activity standards.

Activity coefficients, γ , can be predicted by the Debye–Huckel theory provided the solution is dilute (ionic strength less than about 0.1 M).

$$\log \gamma = -0.5091 z_j^2 \sqrt{\frac{1}{2} \sum C_i z_i^2}, \quad (1)$$

where z_j is the valence of the ion whose activity coefficient is to be determined, and z_i and C_i are the valences and concentrations (M) of any ions in the solution. The square root term is the ionic strength of the solution.

Electrical potential measurements. Deionized mucus samples were placed in dialysis sacs and dialysed to equilibrium against APW. The electrical potential difference between mucus inside the sac and APW outside the sac was measured using reversible calomel electrodes connected to the mucus sample and APW by salt bridges made with agar and saturated KCl. This method for measuring the Donnan potential is recommended by Bull (1964). The Donnan potential arises from the limited mobility of the polyelectrolyte ion and the concomitant deficiency of diffusible ions of like valence. The Donnan potential in an ideal system containing a negatively charged polyelectrolyte and a single uni-univalent salt should equal

$$-\frac{RT}{F} \ln \left[\frac{X^-}{2C} + \sqrt{1 + \left(\frac{X^-}{2C} \right)^2} \right], \quad (2)$$

where C is the external salt concentration and X^- is the concentration of fixed charges, and R , T , and F have their usual meanings.

At equilibrium the chemical potential difference between any ion in mucus and in APW is

$$\mu^m - \mu^o = 0 = RT \ln \frac{a^m}{a^o} + zF(E^m - E^o), \quad (3)$$

where the superscripts m and o refer to the mucus solution and the outside (dialysis) solution, a is ion activity, and z is valence. $E^m - E^o$ is the electrical potential difference between mucus and the dialysis medium. At equilibrium

$$E^m - E^o = \frac{RT}{zF} \ln \frac{a^o}{a^m}, \quad (4)$$

therefore, the measured electrical potential should be equal to the Nernst potential for each ion. The electrical potential was measured directly in mucus samples at equilibrium with APW and was calculated from the Nernst potential of Na in mucus at equilibrium with different NaCl concentrations (1–50 mM).

RESULTS

Ion content of mucus. Data in Table 1, column 1, show that freshly secreted mucus contains high concentrations of Na, Ca, and Cl. Fresh mucus was previously found to resemble the snails' haemolymph in ionic strength and in the concentrations of Na, K, Mg, and Cl (Schlichter, 1981). It was argued that the ion content mainly represents an ultrafiltrate of haemolymph with some contribution from the intracellular medium. When mucus was deionized and dialysed to equilibrium with physiological saline the activity coefficients in mucus were: Na, 0.85; K, 0.74; Ca, 0.34; and Cl, 0.93. Each γ was multiplied by the concentration of the corresponding ion in freshly secreted, undialysed mucus to calculate reasonable ion activities in mucus as it is extruded from a mucus gland. Except for Cl the observed γ values are close to those predicted by Debye-Huckel theory: Na, K and Cl, 0.77; Ca, 0.35. The sum of calculated cation activities in fresh mucus (41.4 mM) is greater than the Cl^- activity (26.4 mM). Therefore, anions such as HCO_3^- and the fixed negative charges on mucus would have to contribute about 15 mM of negative charges to achieve charge neutrality.

Table 1, columns 2 and 3, also show concentrations, activities, and activity coefficients for ions in APW and in mucus at equilibrium with APW. The concentrations of Na, K, and Ca were significantly higher in mucus than in APW ($P < 0.005$, Student's t -test); whereas, Cl was the same ($P > 0.90$). The activities of the Na, K and Cl were significantly higher in mucus than in APW ($P < 0.005$); whereas, the Ca activity was slightly lower in mucus than in APW ($P < 0.075$). In APW the observed activity coefficients for Na, K, and Cl are close to the predicted value of 0.95; whereas, γ_{Ca} is lower than the expected value of 0.82. A similar reduction in γ_{Ca} has been observed in APW by Greenaway (1971) who attributed it to the formation of ion pairs and complexes involving bicarbonate. The sum of cation activities (1.32 mM) exceeds the Cl activity (1.10 mM) by 0.22 mM. Bicarbonate is expected to contribute this amount to maintain charge balance. In mucus at equilibrium with APW, the

Table 1. Ion concentrations, activities, and activity coefficients, (activity/concentration), in mucus and in artificial pond water (APW)

	Freshly secreted mucus	Artificial pond water (APW)	Mucus at equilibrium with APW
Na Concentration	32.6	0.79 ± 0.02 (10)	1.42 ± 0.15 (9)
Activity	27.7	0.75 ± 0.02 (12)	0.91 ± 0.05 (9)
γ	0.85	0.95	0.64
K Concentration	3.6	0.096 ± 0.002 (10)	0.50 ± 0.01 (4)
Activity	2.7	0.092 ± 0.001 (10)	0.24 ± 0.04 (10)
γ	0.74	0.97	0.48
Ca Concentration	16.2	0.38 ± 0.01 (10)	0.48 ± 0.03 (10)
Activity	5.5	0.24 ± 0.01 (10)	0.13 ± 0.07 (4)
γ	0.34	0.63	0.27
Cl Concentration	28.4	1.22 ± 0.02 (10)	1.22 ± 0.03 (20)
Activity	26.4	1.10 ± 0.01 (10)	1.25 ± 0.01 (20)
γ	0.93	0.90	1.02

Values of directly measured variables as mean ± s.e. of the number of samples in parentheses. Concentrations and activities are mM. In freshly secreted mucus the γ for each ion was calculated from ion activity and concentration in mucus dialysed to equilibrium against *Lymanaea* Ringer's. These γ 's were multiplied by ion concentrations in freshly secreted mucus (from Schlichter, 1981) to calculate reasonable values for ion activities in freshly secreted mucus. See text for further explanation. In APW and in mucus at equilibrium with APW the γ values predicted by Debye-Huckel theory (eqn. 1) are Na, K and Cl, 0.95; Ca, 0.82.

γ values for Na, K, and Ca are much lower than predicted; whereas, γ Cl is slightly higher than expected. The difference between the sum of cation activities (1.41 mM) and the Cl activity (1.25 mM) is the expected contribution of HCO_3^- and fixed negative charges to maintain the charge balance.

Titration of fixed acidic groups. Table 2 shows the ion exchange capacity of mucus calculated from titration of fixed acidic groups with K or Ca. The ion exchange capacity (μ -equiv/kg wet mucus) was calculated from these data by taking the water content (99.7%) of mucus dialysed to equilibrium against APW. The measured ion exchange capacity was greater when the acidic groups were titrated with CaCl_2 than with KCl. This suggests that Ca^{2+} forms a tighter complex with the fixed negative groups than does K^+ ; that is, Ca^{2+} displaced more H^+ than did K^+ . A similar result was obtained when Marshall (1978) titrated mucus from a teleost fish, *Leptocottus*. The ion exchange capacity of *Leptocottus* mucus (μ -equiv/g dry mucus) was 69 with KCl and 80 with CaCl_2 . These values are very close to those for *Lymanaea* pedal mucus.

Electrical potential of mucus. The electrical potential difference measured between mucus and the APW dialysis medium, was -2.6 ± 0.7 mV ($n = 9$) with mucus negative to APW. The dialysis tubing had a molecular weight cutoff of 12 000–14 000 and should not have impeded the movement of small ions.

Table 3 shows that deionized mucus samples dialysed to equilibrium with NaCl solutions always had higher Na^+ activities than did the dialysis medium. Nernst potentials for Na calculated from Na activities were all of about the same magnitude (-1.8 to -4.0 mV). These values are similar to the electrical potential between mucus and APW (-2.6 mV) which also agrees reasonably well with the Nernst potential for

Table 2. Titration by *K* and *Ca* of fixed acidic groups on mucus, to determine the ion exchange capacity of mucus

Salt added	pH		Ion exchange capacity	
	Initial	Final	μ -equiv/g dry mucus	μ -equiv/kg wet mucus
3 M-KCl	4.21 \pm 0.02 (19)	3.89 \pm 0.02 (19)	67.4 \pm 3.9 (19)	20.2
0.5 M-CaCl ₂	4.21 \pm 0.02 (19)	3.83 \pm 0.02 (5)	76.4 \pm 6.5 (5)	22.9

Results are as mean \pm s.e. of the number of samples in parentheses. The initial stable pH was measured after CO₂ was driven off by N₂. The final pH was the stable value after excess salt was added. The calculation of exchange capacity as μ -equiv/kg wet weight was from mucus dialysed to equilibrium against APW and having an average concentration of 0.3 g dry mucus/kg wet mucus.

Table 3. *Na* sequestering by mucus

C NaCl (mM)	Na ^o (mM)	Na ^m (mM)	E _{Na} (mV)	[solids] (g dry/kg wet)	X ⁻ Exchange capacity (mEq/kg wet)	Calculated Donnan potential (mV)
1	0.97	1.07	-2.5	0.22	0.015	-0.19
5	4.94	5.39	-2.2	0.40	0.027	-0.07
10	9.16	10.70	-4.0	0.85	0.057	-0.07
15	13.97	15.18	-2.1	1.20	0.081	-0.07
20	19.62	21.05	-1.8	1.32	0.089	-0.06
25	24.14	26.82	-2.7	1.88	0.127	-0.06
50	47.46	51.68	-2.2	3.17	0.214	-0.05

Mucus samples were dialysed against different concentrations (C) of NaCl, Na activities were measured in the dialysis solution (Na^o) and in mucus (Na^m) and Nernst potentials for Na (E_{Na}) were calculated from eqn. (4). The mucus concentration ([solids]) at each NaCl concentration was multiplied by the ion exchange capacity from Table 2 (67.4 \times 10⁻³ m-equiv/g dry) to obtain the ion exchange capacity (X⁻). Corresponding values of X⁻ and C were substituted into eqn. 2 to calculate theoretical Donnan potentials.

Na between mucus and APW (-4.9 mV). These measurements suggest that the electrical potential was sufficient to account for the observed excess Na activity in mucus.

Table 3, column 5, shows a linear relationship between mucus concentration and the ionic strength of the dialysis solution. This reflects osmotic swelling in which the polyelectrolyte molecules appear to contribute little to the osmotic pressure. When the ion exchange capacity measured by KCl titration (Table 2) was corrected for differences in mucus concentration, the calculated values should equal the term, X⁻, of equation (2). The last column of Table 3 shows the Donnan potential calculated from equation 2, which should be approximately equal to the Nernst potential for Na. Possible reasons for the discrepancy will be discussed below. It is not surprising that neither the Donnan nor the Nernst potentials increased with increasing mucus concentration. Increases in ionic strength of the NaCl solution (C) produced the increase in mucus concentration (X⁻) such that the two effects cancelled in equation (2).

DISCUSSION

In *L. stagnalis*, mucus is secreted from single-celled glands onto the integument where it forms a continuous sheet over the entire exposed epidermis (Zylstra, 1972). Freshly secreted mucus contains Na, K, Ca, Mg, and Cl in concentrations equal to or greater than those in haemolymph (Schlichter, 1981). Because these elements are present mainly as free ions they will tend to diffuse into the freshwater environment down their chemical potential gradients. In order for net ion uptake to take place across the integument there must be regions where ions can diffuse from APW through the unstirred mucus layer to the integument. In these regions mucus will affect the concentrations of ions at membrane transport sites through its resistance to diffusion (the classical unstirred layer effect) and through the Donnan potential caused by the glycoprotein polyelectrolyte.

The Donnan equilibrium. When a Donnan equilibrium was established between deionized mucus and a simple salt solution (1–50 mM-NaCl), both the concentrations and activities of Na were higher in the compartment containing the polyelectrolyte. The electrical potential difference between mucus and the dialysis solution should equal the Donnan potential, which in turn must be closely related to the concentration of fixed negative charges in the mucus (eqn. 2). This electrical potential difference should then determine the difference in cation activity between mucus and the dialysis solution (eqn. 4). As expected, the electrical potential difference measured directly between mucus and APW was approximately equal to that calculated from the Nernst equation (eqn. 4) for Na activity in mucus compared with NaCl solutions. In contrast, the ion exchange capacity, measured by titrating the acidic groups, yields too low a value for the Donnan potential (eqn. 2) to account for the excess Na activity in mucus. For example, at equilibrium with a 1 mM solution of NaCl, mucus containing 20 μ molal fixed negative charges (Table 2) should exert a Donnan potential of about -0.25 mV. This is an order of magnitude smaller (less negative) than predicted by the Nernst equation. It is possible that the method of measuring fixed negative charges underestimates their concentration. Tyree (1972) found that when he exposed a negatively charged ion exchanger for several hours to about pH 2, there was an irreversible loss of most of the exchange sites. The system he studied, algal cell walls, contains polygalacturonic acid and may behave in a qualitatively similar manner to snail mucus that contains iduronic acid. It appears that this technique for measuring the ion exchange capacity can greatly underestimate the ability of mucus to sequester cations. Because Marshall (1978) also used this technique, his conclusion that fish mucus sequesters insignificant amounts of cations deserves re-examination.

Unusually low ionic activity coefficients reveal the electrostatic forces between polyelectrolyte ions and small, oppositely charged ions (counterions). The methods used to determine activity coefficients cannot distinguish quantitatively between counterion inactivation by 'ion-binding' and by long-range coulombic interactions (Morawetz, 1975). When the mucus polyelectrolyte solution was at equilibrium with a dilute, complex salt solution (APW), the activity coefficients of Na, K, and Ca were lower than predicted by the Debye-Huckel theory. This suggests coulombic interactions with the fixed charges, and perhaps 'counterion condensation', as described

by Manning (1969), causes most of the lowering of the activity coefficients. Differences between the observed and expected activity coefficients can be ranked to indicate an affinity sequence. Predicted activity coefficients are 0.95 for Na, Cl, and K and 0.82 for Ca. Observed values were 1.02 for Cl, 0.64 for Na, 0.48 for K, and 0.27 for Ca. Therefore, the order of affinities was $\text{Ca} > \text{K} > \text{Na} > \text{Cl}$. Scott (1968) summarized affinity sequences obtained using several techniques on several polyelectrolytes. For polyelectrolytes with mixed sialic and sulphate groups, the affinities were usually $\text{Ca} > \text{K} > \text{Na}$ and sometimes $\text{Ca} > \text{K} \sim \text{Na}$.

When the ionic strength of the complex salt solution was increased (physiological saline), the activity coefficients in mucus approached the values predicted by the Debye-Hückel theory. This suggests that the effects of coulombic interactions and condensation were proportionately reduced, perhaps due to greater charge screening by counterions in the less swollen mucus gel.

Effects of the unstirred mucus layer on uptake kinetics. Unstirred layers are usually thought to reduce the solute concentration at the site of uptake, compared with the concentration in the bulk solution, thereby overestimating the value of the Michaelis constant, K_m (Dietschy *et al.* 1971; Lieb & Stein, 1972; Winne, 1973). Recently, Gains (1980) presented a method for calculating K_m and V_{\max} for solute uptake in the intestine where the thickness of the unstirred layer is not known, requiring only that the thickness be altered by stirring the solution. For the case of an unstirred water layer, Winne (1973) has derived an expression for the solute concentration at the membrane, and for the difference in true and apparent concentrations, which he called the bias term. For simple carrier-mediated uptake, Winne showed that the concentration of a non-electrolyte in the bulk solution overestimates the true Michaelis constant by an amount proportional to the maximum flux and to the thickness of the unstirred layer. This can be written as:

$$C^\circ \text{ (for } \bar{f} = 0.5 V_{\max}) = K_m + 0.5 \frac{V_{\max} \partial}{D} \quad (5)$$

where C° is the solute concentration in the bulk solution in moles/cm³, K_m is the apparent Michaelis constant in moles/cm³, K_m is the apparent Michaelis constant in mole/cm³, ∂ is the thickness of the unstirred layer in cm, and D is the diffusion coefficient of the solute in the unstirred layer in cm²/s.

I will use Winne's equation to show that mucus can reverse the bias term, resulting in an underestimate of K_m . The equations were derived for non-electrolyte transport; whereas, electrolyte diffusion through an unstirred layer depends on the chemical potential gradient which includes both electrical and concentration terms (eqn. 3). Nonetheless, empirically determined properties of snail mucus can be used to calculate the effect of this polyelectrolyte on ion uptake.

In *L. stagnalis* the presence of mucus in the unstirred layer enhances cation concentrations and some cation activities (Tables 1 and 3). At the indistinct interface of the mucus and APW the chemical potentials of ions in the two 'phases' must be equal. The amount by which an unstirred layer of APW can be expected to overestimate the true K_m can be calculated from eqn. (5) using the following data: V_{\max} for Na uptake across *L. stagnalis* integument is 0.225×10^{-6} mol/g animal.h

(Greenaway, 1970). There is about 1 cm^2 of exposed integument per gram animal; therefore, V_{max} is about $6 \times 10^{-11} \text{ mol/cm}^2 \cdot \text{sec}$. As an estimate of D for Na in mucus, the value measured by Marshall (1978) for fish mucus can be used; $1 \times 10^{-5} \text{ cm}^2/\text{s}$. A reasonable range for the mucus thickness on *L. stagnalis* integument is $10\text{--}500 \mu\text{m}$. For these thicknesses, the bias term ($0.5 V_{\text{max}} \partial/D$) from eqn. (5) ranges from 3.1×10^{-8} to $1.5 \times 10^{-4} \text{ mol/l}$. Mucus enhances the cation concentrations by 6.3×10^{-4} , 4.0×10^{-4} , and $1.0 \times 10^{-4} \text{ mol/l}$ for Na, K and Ca, respectively. This enhancement will be more than enough to compensate for the bias term in eqn. (5). In other experimental systems, the bias term may be larger because of a larger V_{max} ; however, the ion-sequestering capacity of mucus may also be greater in other systems. The nature of the mucus layer in each experimental system needs to be considered.

An implicit assumption in calculating the true K_m from the concentration at the membrane is that the charge distribution in mucus is homogeneous at the membrane surface. This is usually assumed to be a reasonable approximation for swollen polyelectrolytes in dilute solutions (Morawetz, 1975). However, at the molecular level we do not know what ion concentrations or activities the ion carriers 'see'.

For any sub-maximal ion uptake rate in *L. stagnalis*, the carriers may require a lower affinity in the presence of mucus than in its absence. This could be advantageous to a freshwater animal living in an environment more dilute than the saturation concentration. For example, *L. stagnalis* lives in freshwaters that may have about the same Na concentration as the 0.25 mM determined by Greenaway (1970) to be the K_m for uptake. In such dilute waters the true K_m may be two or more times greater than this value. The resulting decrease in the affinity constant could facilitate unloading of the ion carrier at the inside of the membrane.

I am grateful to Drs J. Dainty, J. Machin, and M. T. Tyree for discussions and for helpful criticisms of this manuscript. This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) grants to Drs Dainty and Machin, and an NSERC postgraduate scholarship to the author.

REFERENCES

- BULL, H. B. (1964). *An Introduction to Physical Biochemistry*, p. 433. Philadelphia: F. A. Davis.
- CARRIKER, M. R. (1946). Observations on the functioning of the alimentary system of the snail *Lymnaea stagnalis* appressa Say. *Biol. Bull. mar. biol. Lab., Woods Hole* **91**, 88-111.
- DAINTY, J. & HOUSE, C. R. (1966). 'Unstirred layers' in frog skin. *J. Physiol., Lond.* **182**, 66-78.
- DE WIT, N. D., WITTEVEEN, J. & VAN DER WOUDE, H. A. (1980). Integumental Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchanges in the freshwater snail *Lymnaea stagnalis*. *Proc. K. ned. Akad. Wet. Series C* **83**, 209-215.
- DIETSCHY, J. M., SALLEE, V. L. & WILSON, F. A. (1971). Unstirred water layers and absorption across the intestinal mucosa. *Gastroenterology* **61**, 932-934.
- GAINS, N. (1980). The determination of the kinetic parameters of a carrier mediated transport process in the presence of an unstirred water layer. *J. theor. Biol.* **87**, 559-568.
- GREENAWAY, P. (1970). Sodium regulation in the freshwater mollusc *Limnaea stagnalis* (L.) (Gastropoda: Pulmonata). *J. exp. Biol.* **53**, 147-163.
- GREENAWAY, P. (1971). Calcium regulation in the freshwater mollusc, *Limnaea stagnalis* (L.) (Gastropoda: Pulmonata). I. The effect of internal and external calcium concentration. *J. exp. Biol.* **54**, 199-214.
- HELFFERICH, F. (1962). *Ion Exchange*, p. 624. New York: McGraw-Hill.
- LIEB, W. R. & STEIN, W. D. (1972). The influence of unstirred layers on the kinetics of carrier-mediated transport. *J. theor. Biol.* **36**, 641-645.

- MANNING, G. S. (1969). Limiting laws and counterion condensation in polyelectrolyte solutions. I. Colligative properties. *J. chem. Phys.* **51**, 924-933.
- MARSHALL, W. S. (1978). On the involvement of mucous secretion in teleost osmoregulation. *Can. J. Zool.* **56**, 1088-1091.
- MORAWETZ, H. (1975). *Macromolecules in Solution*, pp. 369-375. New York: Wiley.
- OVERBEEK, J. TH. G. (1956). The Donnan equilibrium. *Prog. Biophys.* **6**, 58-84.
- SCHLICHTER, L. (1981). Ion relations of haemolymph, pallial fluid and mucus of *Lymnaea stagnalis*. *Can. J. Zool.* **59**, 605-613.
- SCOTT, J. E. (1968). Ion binding in solutions containing acid mucopolysaccharides. In: *The Chemical Physiology of Mucopolysaccharides* (ed. G. Quintarelli), pp. 171-187. Boston: Little, Brown.
- TYREE, M. T. (1972). Ion exchange and the transport of water and solutes in Characean cell walls. Ph.D. thesis, Churchill College, Cambridge, England.
- VAN DER BORCHT, O. & VAN PUymbROECK, S. (1964). Active transport of alkaline earth ions as physiological base of the accumulation of some radionuclides in freshwater molluscs. *Nature, Lond.* **204**, 533-534.
- WINNE, D. (1973). Unstirred layer, source of biased Michaelis constant in membrane transport. *Biochim. biophys. Acta* **298**, 27-31.
- ZYLSTRA, U. (1972). Histochemistry and ultrastructure of the epidermis and the subepidermal gland cells of the freshwater snails *Lymnaea stagnalis* and *Biomphalaria pfeifferi*. *Z. Zellforsch. mikrosk. Anat.* **130**, 93-134.