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Reflection Coefficient and Permeability of Urea in the Proximal Convolution of the Rat Kidney

An Application of Non-Equilibrium Thermodynamics for a Multicomponent System with Active Transport

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Summary. The transport theory of Kedem and Katchalsky which was derived for passive transport in a two-compartment system is generalized for a multicomponent system with active transport, so that it can be applied to more complicated biological membranes.

Equations have been derived to describe the transport of urea through the proximal convolution of the rat kidney and the permeability and the reflection coefficient have been determined. The permeability coefficient ($\tilde{P}_u$) measured with the microperfusion and stop flow microperfusion methods, was found to be 6.0 and 5.2×10^{-5} mm²/sec, respectively.

The reflection coefficient (σ) was determined in a stationary state situation and found to be 0.68. Earlier free flow micropuncture results together with the P_u and σ_u of this study indicate that 50% of the filtered urea is reabsorbed proximally and that approximately half of this amount is reabsorbed by solvent drag and the rest by diffusion.

In the Appendix, a theoretical treatment of nonelectrolyte transport in a multicomponent system with active transport is given.

Many attempts have been made to describe the material-transport across biological membranes by the thermodynamics of irreversible processes. Beside the permeability coefficient, the reflection coefficient, which was derived by Staverman [18] and applied to a passive two-component system of electrolytes by Kedem and Katchalsky [6] has proven to be very useful. For a multicomponent system with active transport, which applies to many biological membranes, the theory has to be generalized. This is done in the Appendix to this paper. The main features of the extended theory are described by the following equations:

For the volume flow (J_V) one gets

$$J_V = L_{VV}\left(X_V - \sum_{i=1}^{n} \sigma_i \bar{c}_i X_i\right) + L_{VE}E + L_{VA}A'. \qquad \text{(A.9)}$$

X_V, X_i are the driving forces for the volume flow and the i-components respectively, E is the electrical potential difference, A' is the affinity of the chemical reaction, σ_i is the reflection coefficient and $\bar{c}_i$ the average concentration across the membrane, L_{VV}, L_{VE}, and L_{VA} are coupling coefficients.

For the flow of the i-th component (J_i) one gets

$$J_i=(1-\sigma_i)\bar{c}_i J_V+\sum_{i=1}^{n} L_{ik}^* X_k+L_{iE}^* E+L_{iA}^* A'. \tag{A.10}$$

X_k is the driving force for the K component, L_{iK}^*, L_{iE}^*, L_{iA}^* are coupling coefficients where the * marks a reference point relative to volume flow.

These equations become more simple in the case of dilute solutions near equilibrium. Then we have the equations

$$J_V=-L_{VV}RT\left(\frac{\Delta P}{RT}-\sum_{j=n+1}^{m}\Delta c_j-\sum_{i=1}^{m}\sigma_i\Delta c_i\right)+L_{VE}E+L_{VA}A' \tag{A.17}$$

where ΔP is the hydrostatic pressure difference, Δc_j the concentration difference of an impermeable solute j and Δc_i that of a permeable solute i across the membrane, and

$$J_i=(1-\sigma_i)\bar{c}_i J_V-\tilde{P}_i\Delta c_i+L_{iE}^* E+L_{iA}^* A'$$

where $\tilde{P}_i$ is the tracer permeability coefficient per unit length of the i-th component. In this form the equation can be applied to the kidney tubules. At the quasi stationary state of the shrinking droplet [4] one derives the equation

$$-\sigma_i\bar{c}_i J_V-\tilde{P}_i\Delta c_{i\infty}+L_{iE}^* E+L_{iA}^* A'=0. \tag{A.20}$$

$\Delta c_{i\infty}$ is the concentration difference across the membrane in the stationary state. Eq. (A.20) then becomes

$$\sigma_i=-\frac{\tilde{P}_i}{J_V}\frac{\Delta c_{i\infty}}{\bar{c}_i} \tag{A.21}$$

in the case of nonelectrolytes with no active transport of the i-th component. Eq. (A.21) is identical with an equation for a purely passive system except that J_V of Eq. (A.21) is the total volume flow (active plus passive). If any of the assumptions made in the derivation of Eq. (A.21) is not fulfilled, the equation is not valid and the determination of σ_i becomes much more complicated.

Eq. (A.21) was used for estimating σ urea for the proximal convolution. The transtubular volume flux J_V was varied and Δc and $\bar{c}$ were measured. $\tilde{P}$ was determined with $^{14}C_{urea}$ in an independent series of experiments where J_V was kept at zero. The values found with these procedures were 6.0 and 5.2×10^{-5} mm^2/sec for $\tilde{P}_{urea}$ and 0.68 for σ_{urea}.

Materials and Methods

All our experiments were performed on male Wistar rats with body weights of 150 to 250 g. The procedure of preparing the animals for micropuncture [19], the method of the shrinking droplet [4] and of continuous microperfusion [16] have been described in detail elsewhere. During experiments, the appropriate peritubular capillaries were perfused with artificial solutions as published by Frömter [2] and by Spitzer and Windhager [17]. All studies were performed at the same luminal diameter [10]. A meniscus correction factor of one tubular diameter was used with the shrinking droplet technique.

Measurement of the Permeability Coefficient ($\tilde{P}_u$) (Experimental Groups 5 and 5a)

In one experimental group (no. 5) the permeability coefficient for urea ($\tilde{P}_u$) was measured with the continuous microperfusion method described first by Sonnenberg, Deetjen and Hampel [16]. Transtubular volume flux was brought to zero by adding 40 mM raffinose to the intratubular solution (Table 1). This solution also contained 5 mM of ^{14}C urea with a specific activity of 50 mC/mmole. The capillary perfusate contained 5 mM unlabeled urea. The luminal perfusion rate ($\dot{V}$) was 5 nl/min and the contact time of the perfusate with the tubular wall 0.25 to 10.0 sec. During the perfusion, the luminal diameter was kept at 30 μ by applying a corresponding counter pressure. The urea concentration of the reaspirated perfusate was evaluated by counting

Table 1. Solutions for inducing different volume fluxes (J_v) and measuring the urea permeability of the proximal convolution

Composition	Experimental group 1		Experimental group 2		Experimental group 3		Experimental group 4		Experimental groups 5 and 5a	
	Luminal perfusion	Capillaries	Luminal perfusion	Capillaries	Luminal perfusion	Capillaries	Luminal perfusion	Capillaries	Luminal perfusion	Capillaries
NaCl (mEquiv)	110	103	103	103	103	78	145	95	120	105
$NaHCO_3$ (mEquiv)	—	35	35	35	35	35	—	35	—	35
Na-acetate (mEquiv)	10	10	10	10	10	10	10	10	10	10
$CaCl_2$ (mEquiv)	3	3	3	3	3	3	—	3	3	3
Raffinose (mM)	50	—	—	—	—	50	—	36	40	—
^{14}C-urea (mM)	5	5	6.0–7.5	5	6.0–7.5	5	6.0–7.5	5	5–25	—
Urea (mM) (unlabeled)	—	—	—	—	—	—	—	—	—	5–25

^{14}C activity in a liquid scintillation counter. The half length ($l_{1/2}$) for the disappearance of the transtubular ^{14}C urea concentration gradient was determined by drawing a regression line through a plot of all the data and $\tilde{P}$ was calculated by the equation:

$$\tilde{P}_{\text{urea}} = \frac{0.693 \cdot \dot{V}}{l_{1/2}}. \tag{1}$$

In a second experimental group (no. 5a), $\tilde{P}_u$ was measured with the stop flow microperfusion method. The test solutions had the same composition as in experimental group 5 except that their urea concentration was 25 mM. In the luminal solution, part of the urea was ^{14}C labeled. The luminal test solution was injected into an oil blocked proximal tubule and aspirated after 4 and 10 sec for ^{14}C urea counting. For each contact time the average concentration in the aspirate $c_{\tau 1}$ and $c_{\tau 2}$ was calculated and $\tilde{P}_{\text{urea}}$ evaluated by the equation:

$$\tilde{P} = \frac{r^2 \pi}{\Delta \tau} \ln \frac{c_{\tau_1}}{c_{\tau_2}}. \tag{2}$$

$\Delta \tau$ is the difference in contact time for the two series of samples.

Measurement of $\frac{\Delta c_u}{J_v \cdot \bar{c}_u}$ *(Experimental Groups 1 through 4)*

Volume flow out of the tubule (J_v) was determined by the shrinking droplet technique of Gertz [4]. The half time disappearance ($\tau_{1/2}$) (i.e., the time during which the volume of the droplet decreases by 50%) was derived graphically from series photographs and J_v was calculated according to the equation:

$$J_v = \frac{0.693 \cdot \pi r^2}{\tau_{1/2}}. \tag{3}$$

The luminal radius (r) in these determinations had the same size as the radius (r) in the urea permeability determinations. Transtubular volume flow was varied by changing the bicarbonate concentration [3] or by adding raffinose to the perfusate of the peritubular capillaries (Table 1). With each perfusate a series of J_v determinations and a separate series of Δc measurements were performed and means as well as SE values calculated. At least 6 animals were investigated in each group; the number of experiments is listed in Table 2, together with the calculated mean and SE values. In experimental groups 2 through 4 the stationary urea concentration was measured in samples recollected from the intratubular droplet after a shrinkage time of 20 to 30 sec. This time interval corresponds to about three to five diffusion half times ($\tau_{1/2}$) and the intratubular urea concentration had thus approached its stationary concentration. To facilitate the approach to stationary state concentration, the amount of urea in the solution injected into the tubule was adjusted so that it was similar to the concentration expected in the stationary state (Table 1).

In experimental group no. 1 the steady state concentration for urea was measured under the condition of zero net volume flux. This was achieved by adding an equivalent amount of raffinose to the luminal solution.

Since capillary perfusate and intratubular solutions contained $^{14}C_{\text{urea}}$ of the same specific activity, the urea concentrations of all samples could be evaluated by ^{14}C counting.

Table 2. Results of the measurements of volume flow and urea concentration at steady state[a]

Experimental group	J_v ($\times 10^{-5}$ mm^2 · sec^{-1})	$\tau_{1/2}$ (sec)	$\bar{c}$ (mM)	Δc (mM)	$\bar{c}_u \cdot J_v$ ($\times 10^{-11}$ mmoles · mm^{-1} · sec^{-1})
1	0	∞	4.93 ± 0.16 $n=17$	-0.15 ± 0.08 $n=17$	0
2	1.58 ± 0.1 $n=27$	31.0 ± 2.2 $n=27$	5.33 ± 0.03 $n=55$	0.65 ± 0.06 $n=55$	8.43 ± 0.50
3	2.30 ± 0.14 $n=36$	21.3 ± 1.6 $n=36$	5.78 ± 0.08 $n=38$	1.55 ± 0.16 $n=38$	13.28 ± 0.83
4	3.14 ± 0.1 $n=33$	15.6 ± 0.7 $n=33$	6.05 ± 0.09 $n=34$	2.10 ± 0.17 $n=34$	19.02 ± 0.67

[a] The mean value $\pm$ SE and the number of experiments are noted for J_v, $\tau_{1/2}$, $\bar{c}$, Δc, and $c \cdot J_v$ for experimental groups 1 through 4.

Results

In Fig. 1 the change of the transtubular urea concentration difference is plotted against the length of the perfused tubular segment. From the regression line through the 50 single measurements a mean $l_{1/2}$ of 960 µ was found. From this and a perfusion rate of 5 nl/min a $\tilde{P}_{urea}$ value of 6.0×10^{-5} mm^2/sec was calculated using Eq. (1). In the stop flow micro-

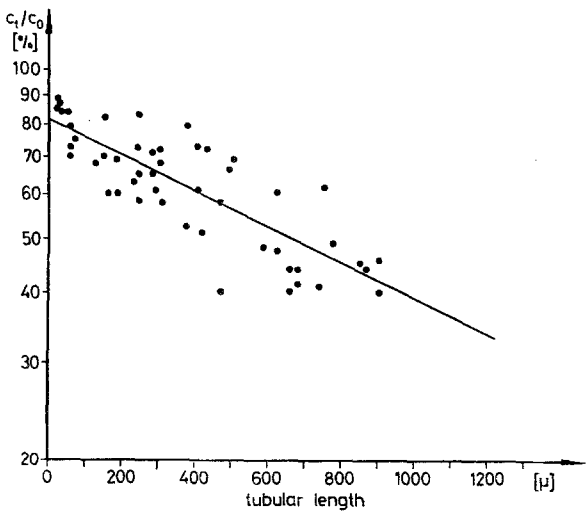

Fig. 1. Decay of the transtubular urea concentration difference with tubule length. The microperfusion method with zero transtubular volume flux and simultaneous capillary perfusion was applied. The regression line is calculated according to the method of least squares and indicates an absorption half length of 960 µ

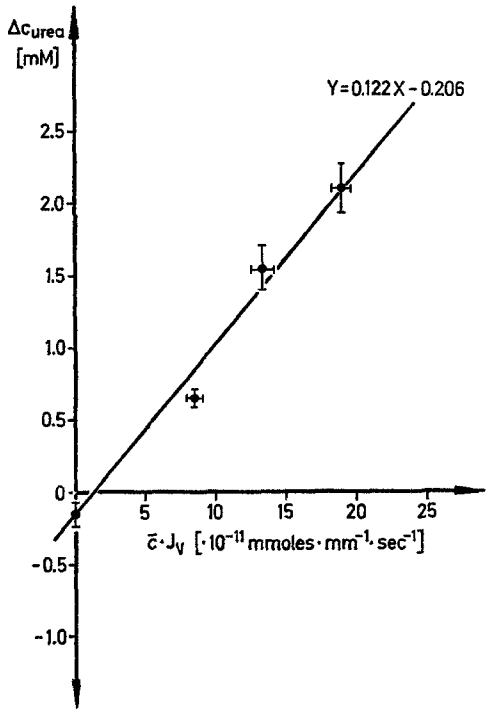

Fig. 2. Plot of the stationary transtubular concentration difference as measured in a shrinking droplet against the transtubular volume flux J_v multiplied by the mean urea concentration $\bar{c}$ across the tubule

perfusion series a total of 61 samples was collected; approximately half were collected after 4 sec (τ_1) and the other half after 10 sec (τ_2). From the mean of these data a $\tilde{P}_{\text{urea}}$ of 5.2×10^{-5} mm^2/sec was calculated using Eq. (2). Fig. 2 shows a plot of Δc_{urea} against $\bar{c} \cdot J_V$. The mean values for each group are on a straight line which comes very close to the "0" intersection of abscissa and ordinate. Inserting the mean of the two $\tilde{P}$ values and the $\frac{\Delta c}{\bar{c} J_v}$ value from Fig. 2 into Eq. (A.21) a σ value for urea of 0.68 was found.

Discussion

The Method

Until recently, permeability as well as transtubular water flux was expressed in terms of the area of a cylinder with a radius equal to that of the tubular lumen [19]. This may be misleading since it was found in a recent study that transtubular isotonic absorption as well as ^{36}Cl permeability is independent of the luminal diameter and almost independent

of the perfusion rate [11]. Therefore, in this study we expressed both parameters per tubular length.

Another question is whether the length of the shrinking droplet in the Gertz technique should be measured from the tops of the oil menisci or from the edges. In many cases we have found that it is only the edge distance that gives a straight line when expressed as a semilogarithmic plot against shrinkage time, and this has also been confirmed by A. Z. Györy (*personal communication*). Because it is easier to measure the distance between the tops of the two menisci, we have taken this measurement and made a correction by adding to it the diameter of the tubule. This gave a reasonable estimate of the actual edge to edge distance since the oil meniscus is apparently hemispherical. The isotonic fluid absorption measured with the shrinking droplet method is about 30% smaller than that found in microperfusion experiments [11]. The reason for this discrepancy is unclear. It does not, however, interfere with our σ measurement because Δc and J_V were measured under exactly the same experimental conditions. But it should be emphasized that each calculated value of σ strictly applies only to the experimental conditions under which measurements were made.

The further experimental problem concerns the question of whether stationary conditions may be considered to apply to the shrinking droplet experiments. In a shrinking droplet experiment with $\tau_{1/2}$ in the range of 10 to 15 sec it is only possible to get enough tubular fluid for analysis after 20 to 30 sec contact time when the samples are pooled. A diffusion half time ($\tau_{1/2}$) of about one-fourth of the contact time should, however, be satisfactory to reach a true steady state. The same will be true for other substances with permeability coefficients in the same range as for instance Cl^- ions. Application of the methods used in this study may be more problematic for substances with a permeability lower than that of urea.

The values for urea permeability which were obtained, using artificial capillary perfusion with both the microperfusion and stop flow methods, were almost identical. In both series of experiments the luminal diameter was maintained at 30 μ. These values nevertheless, also coincide with a $\tilde{P}_{urea}$ value of 5.3×10^{-5} mm²/sec obtained from microperfusion studies in which blood was flowing through the capillaries and the luminal diameter was maintained at 20 μ [20]. These findings show that with urea, also, permeability is independent of the luminal radius as was observed originally in a microperfusion study with ^{36}Cl [11]. Furthermore, the luminal brush border membrane must be rate limiting or urea must preferentially pass through a paracellular shunt path. Otherwise the $\tilde{P}_{urea}$ value obtained with the stop flow microperfusion method would have been larger than that found

with the continuous microperfusion method. Indeed, electrophysiological measurements point to a preponderance of the paracellular shunt path for passive ion permeation [3]. Using a mean transtubular $\tilde{P}_{urea}$ of 5.6×10^{-5} mm^2/sec, a σ_{urea} of 0.68 was calculated using Eq. (A.21) in this study. Previously, σ_{urea} of the proximal convolution of the rat had been evaluated by us by quite a different method [20]. By perfusing the tubule with solutions of different nonelectrolyte concentrations it was possible to compare the ability of urea to induce transtubular water flux with that of raffinose which has a reflection coefficient of "one". In these experiments it was found that a concentration of 254 mosm/liter urea was equivalent to 200 mosm/liter raffinose, from which a reflection coefficient of 0.79 was estimated for urea. This is somewhat higher than the value of 0.68 presented in this study. Since we had used quite different solutions at each site of the tubular wall in the previous "osmotic" study the system may have been outside the range of linearity which – in a strict sense – exists only near equilibrium.

What conclusion can be drawn from the $\tilde{P}_{urea}$ and σ_{urea} data for the outflux of urea during the normal free flow situation? Endproximal TF/P_{urea} divided by the TF/P_{inulin} ratio is 0.5 [8, 21] indicating that under free flow conditions 50% of the filtered urea is reabsorbed in the proximal convolution. Using this figure, a $\bar{c}_{urea}$ value of 1.125 times the plasma concentration, the $\tilde{P}$ and σ values reported in this paper, and an endproximal $\frac{TF}{P}$ inulin of 3 one can estimate that 45% of the proximal urea absorption is due to solvent drag and 55% is due to diffusion. With the earlier measured σ_{urea} of 0.79 the ratio would be 30% reabsorbed by solvent drag and 70% by diffusion. An active urea transport could not be demonstrated in the present study of the proximal tubule since in the experimental situation when J_v and Φ_s were zero (Table 2, group 1) it was also found that Δc did not deviate significantly from zero. The transport of urea could therefore be completely described by Eq. (A.9).

We thank Mrs. S. Klöss for her expert technical assistance.

Appendix

Application of Non-Equilibrium Thermodynamics to Urea Transport[1]

Before we apply non-equilibrium thermodynamics to kidney tubule transport, we have to generalize the formalism of Kedem and Katchalsky [6]

1 By F. Sauer. A summary was presented at the International Colloquy 'Urea and the Kidney' held at Sarasota, Florida, 1968.

and of Smit [15] for multicomponent systems with active transport. The main purpose of this Appendix is the generalization of Staverman's reflection coefficient for this kind of system. Only the basic steps of the theory are presented here. A more detailed description will be published elsewhere.

The starting point of our consideration is the standard membrane system of two liquid phases ′ and ″ separated by a membrane in a steady state. Each of the liquid phases should be homogeneous and in internal equilibrium. The components of each phase should be the water (index W) and n solute components (index i), which can permeate across the membrane. In addition we allow the presence of $m-n$ impermeable solute components (index j). For the thermodynamics of our system we are choosing electroneutral solute components. This means every component is a salt or a nonelectrolyte.[2] To describe the system when ions are present, *see* Schlögl [14]. From the Gibbs equation we derive an expression for the entropy-production in the case $\Delta T=0$. Use has been made of the steady state conditions.

One gets

$$T\frac{dS\,\mathrm{int}}{dt}=-J_W\Delta\mu_W-\sum_{i=1}^{n}J_i\Delta\mu_i+IE+J_cA'. \tag{A.1}$$

Here $J_W\equiv\frac{dn''_W}{dt}$ is the water flow relative to the membrane into the ″ phase and $\Delta\mu_W=\mu''_W-\mu'_W$ is the difference of the chemical potential of water across the membrane. J_i and $\Delta\mu_i$ are the flows and differences of the chemical potentials of the n permeating solute components. I is the total electrical current and E the electrical potential difference between the phases measured by identical reversible electrodes. J_c is the chemical production rate inside the membrane and A' the affinity of the chemical reaction on the ′ side of the membrane. For simplicity we have assumed only one reaction. The problem can easily be generalized for the case of more than one reaction. If i is a solute component involved in the chemical reaction, then we must discriminate between the flow J_i of the component on the ″ side of the membrane and the flow J'_i on the ′ side. We have the relation

$$J_i+J'_i=\nu_iJ_c \tag{A.2}$$

where ν_i is the stoichiometric number of the i-th component.

For membrane systems near equilibrium it is convenient to change the frame of reference for the flows of water and the solute components. Instead

2 One should make the remark that there is a certain arbitrariness by introducing salts into the thermodynamic picture. *See*, for example, Fitts [1].

of J_W and the J_i we introduce

$$J_i^* \equiv J_i - \bar{c}_i J_v \quad \text{and} \quad J_v = V_W J_W + \sum_{i=1}^{n} V_i J_i. \tag{A.3}$$

J_v is the volume flow and J_i^* the flow of the i-th component relative to the volume flow. V_i and V_W are the partial molar volumes and $\bar{c}_i$ a suitable chosen average concentration. Near equilibrium one can choose $\bar{c}_i = \frac{1}{2}(c_i' + c_i'')$.

Elimination of J_i and J_W in Eq. (A.1) with help of Eq. (A.3) yields

$$T \frac{dS\,\mathrm{int}}{dt} = J_V X_V + \sum_{i=1}^{n} J_i^* X_i + IE + J_c A', \tag{A.4}$$

with

$$\begin{aligned} X_V &= -\left(\Delta P - \sum_{j=n+1}^{m} \bar{c}_j \left(\Delta \mu_j - \frac{V_j}{V_W} \Delta \mu_W \right) \right) \\ X_i &= -\left(\Delta \mu_i - \frac{V_i}{V_W} \Delta \mu_W \right). \end{aligned} \tag{A.5}$$

X_V is the driving force conjugated with the volume flow. One should notice that in X_V appears the pressure difference ΔP and the differences of the chemical potentials of the $m-n$ non-permeating components. X_i the driving force conjugated with J_i^* is independent of ΔP.

Near equilibrium we expect linear laws existing between flows and forces. For our choice of flows and forces they have the form

$$\begin{aligned} J_V &= L_{VV} X_V + \sum_{i=1}^{n} L_{V_i} X_i + L_{VE} E + L_{VA} A' \\ J_i^* &= L_{iV} X_V + \sum_{k=1}^{n} L_{ik} X_k + L_{iE} E + L_{iA} A' \\ I &= L_{EV} X_V + \sum_{k=1}^{n} L_{Ek} X_k + L_{EE} E + L_{EA} A' \\ J_c &= L_{AV} X_V + \sum_{k=1}^{n} L_{Ak} X_k + L_{AE} E + L_{AA} A'. \end{aligned} \tag{A.6}$$

Because of the idea of linear laws the L-coefficients should not depend on the driving forces X_V, X_i, E, and A' but they may depend on the average state of the membrane system, for example, $\bar{c}$. If there is $L_{iA} \neq 0$ for a component i which is not involved in chemical reactions we say i is transported actively relative to the volume flow. Notice that the phenomena of active transport depends on the frame of reference. If $L_{VA} \neq 0$ we have active volume flow.[3]

3 The introduction of a separate active volume flow into the thermodynamic description does not exclude the possibility that this part of the volume flows depends on other active flows.

For our choice of flows and forces the L-coefficients obey Onsager-relations

$$\begin{aligned} &L_{iV}=L_{Vi}; \quad L_{VE}=L_{EV}; \quad L_{VA}=L_{AV} \\ &L_{ik}=L_{ki}; \quad L_{iE}=L_{Ei}; \quad L_{iA}=L_{Ai} \\ &L_{EA}=L_{AE}. \end{aligned} \tag{A.7}$$

We will now generalize the idea of Staverman's reflection coefficient for a multicomponent system with active transport. Instead of L_{iV} we introduce the reflection coefficient σ_i with the help of the definition

$$\bar{c}_i \sigma_i = -\frac{L_{iV}}{L_{VV}}. \tag{A.8}$$

We get then

$$J_V = L_{VV}\left(X_V - \sum_{i=1}^{n} \sigma_i \bar{c}_i X_i\right) + L_{VE}E + L_{VA}A' \tag{A.9}$$

$$J_i^* = \sigma_i \bar{c}_i J_V + \sum_{i=1}^{n} L_{ik}^* X_k + L_{iE}^* E + L_{iA}^* A'$$

or

$$J_i = (1-\sigma_i)\bar{c}_i J_V + \sum_{i=1}^{n} L_{ik}^* X_k + L_{iE}^* E + L_{iA}^* A' \tag{A.10}$$

with

$$L_{ik}^* = L_{ik} - \frac{L_{iV}L_{kV}}{L_{VV}}; \quad L_{iE}^* = L_{iE} - \frac{L_{iV}L_{EV}}{L_{VV}} \tag{A.11}$$

$$L_{iA}^* = L_{iA} - \frac{L_{iV}L_{AV}}{L_{VV}}.$$

One should notice, that in Eqs. (A.9) and (A.10) active transport gets a different meaning, because we have eliminated part of the active volume flow from the flows of the i-th component. From Eq. (A.11) we see that L_{iA}^* does not necessarily vanish when $L_{iA}=0$ if L_{iV} and L_{AV} are unequal zero. From the definition of X_V we see that it can be written in the form

$$X_V = -\Delta P - \sum_{j=n+1}^{m} c_j X_j \tag{A.12}$$

with

$$X_j = -\left(\Delta\mu_j - \frac{V_j}{V_W}\Delta\mu_W\right) \tag{A.13}$$

the driving force for the impermeable components. Putting Eq. (A.12) into Eq. (A.10) we notice that the reflection coefficient for impermeable components is one,

$$\sigma_j = 1 \quad (j=n+1 \ldots m), \tag{A.14}$$

in agreement with the general idea of reflection coefficients. Eqs. (A.8), (A.9) and (A.10) give the required generalization of Staverman's reflection coefficient for multicomponent systems with active transport. Before applying them to kidney tubule transport they should be simplified. This can be done in the case of dilute solutions for a membrane system near equilibrium. We have dilute solutions, when the following conditions are fulfilled,

$$\bar{c}_i V_i \ll 1 \quad (i=1 \ldots n); \quad \bar{c}_j V_j \ll 1 \quad (j=n+1 \ldots m)$$

and

$$\sum_{i=1}^{n} \bar{c}_i V_i + \sum_{j=n+1}^{m} c_j V_j \ll 1. \tag{A.15}$$

The system is near equilibrium when

$$\frac{\Delta c_i}{c_i} \ll 1 \quad (i=1, \ldots, n); \quad \frac{\Delta c_j}{c_j} \ll 1 \quad (j=n+1, \ldots, m). \tag{A.16}$$

Under these conditions Eqs. (A.9) and (A.10) reduce to

$$J_V = -L_{VV} RT \left(\frac{\Delta P}{RT} - \sum_{j=n+1}^{m} \Delta c_j - \sum_{i=1}^{n} \sigma_i \Delta c_i \right) + L_{VE} E + L_{VA} A'$$

and (A.17)

$$J_i = (1-\sigma_i) \bar{c}_i J_V - \tilde{P}_i \Delta c_i + L^*_{iE} E + L^*_{iA} A'.$$

R is the gas constant and T the absolute temperature. $\tilde{P}_i$ is the permeability of the i-th component. By deriving Eq. (A.17) we have neglected all higher order terms in Δc and assumed that the coupling coefficients L_{ik} $(i \neq k)$ are small compared to L_{ii}, so that we can neglect them too.

We now apply Eq. (A.17) to the shrinking drop experiment. This is possible when we assume that the membrane is in a steady state all the time. We call the luminal phase the '' phase. For the change of concentration c_i'' we get from mass-balance equations

$$\frac{dc_i''}{dt} = \frac{1}{V''} (J_i - c_i'' J_V). \tag{A.18}$$

In our approximation we can substitute c_i'' by c_i in the right-hand side of Eq. (A.18) and get

$$\frac{dc_i''}{dt} = \frac{1}{V''} (J_i - \bar{c}_i J_V). \tag{A.19}$$

Now let us discuss a quasi stationary state of the lumen with $\frac{dc_i''}{dt} \approx 0$. This means the concentrations of all the components should not change with time. Then we get from Eqs. (A.19) and (A.17)

$$-\sigma_i \bar{c}_i J_V - \tilde{P}_i \Delta c_{i\infty} + L^*_{iE} E + L^*_{iA} A' = 0. \tag{A.20}$$

Here Δc_i is the quasi-stationary concentration difference. For nonelectrolytes L_{iE}^* is mostly zero. If, in addition, L_{iA}^* is found to be zero, we get from Eq. (A.20)

$$\sigma_i = -\frac{\tilde{P}_i}{J_V}\frac{\Delta c_{i\infty}}{\bar{c}_i}. \tag{A.21}$$

This formula tells us that for nonelectrolytes with vanishing L_{iA}^* we can calculate σ_i, when we measure $\tilde{P}_i$, J_V and $\Delta c_{i\infty}$, also when there is active volume flow. In dilute solutions, where one can neglect tracer coupling, we can substitute $\tilde{P}_i$ by the tracer permeability per unit length for the i-th component.

If one of the assumptions we made is not fulfilled, then Eq. (A.21) is not valid and the determination of σ_i becomes much more complicated.

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