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SODIUM CHLORIDE REFLECTION COEFFICIENT IN RABBIT GALL BLADDER

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SUMMARY

By means of the Kedem-Katchalsky thermodynamic description of active transport, a relationship has been derived between the apparent reflection coefficient and the Staverman reflection coefficient for passive transport of a solute which is both actively and passively transported. The relationship between volumetric flow and its driving forces, containing the Staverman reflection coefficient, was tested for sodium chloride in rabbit gall bladder and the reflection coefficient was evaluated.

INTRODUCTION

Mass transfer across a membrane has been described by means of linear phenomenological equations relating solvent and solute flows to the chemical potential difference of each species present in the two compartments separated by the membrane. Making a change of flow and force coordinates, also volumetric (J_V) and diffusional (J_D) flows can be also linearly correlated with hydrostatic (ΔP) and osmotic ($\Delta\Pi$) pressure differences across the membrane [1]. Such a phenomenological description accounts for the non-ideal selectivity of actual membranes by means of the reflection coefficient σ, whose relationship with the permeability and osmotic flow coefficients L_P and L_{PD} which appear in the phenomenological equations is given by

$$\sigma = \left(\frac{\Delta P}{\Delta \pi}\right)_{J_V = 0} = -\frac{L_{PD}}{L_P} \qquad (1)$$

In physiological studies of transport across biological membranes, the reflection coefficient is usually determined, according to Eqn 1, measuring the hydrostatic pressure difference which zeroes volumetric flow at a given partial osmotic pressure difference of the considered solute. Hydrostatic pressure difference is often simulated by means of an equal opposite osmotic pressure difference of a non-permeating nonelectrolyte [23, 24].

Following Kedem's suggestion [6], solute and water flows (J_S and J_W) in an actively transporting membrane are coupled to the flow of a chemical reaction J_R, whose driving force is the chemical reaction affinity A_R. Thus the dissipation function

(Φ) for the isothermal membrane solutions system can be written as

$$\Phi = J_W \Delta\mu_W + J_S \Delta\mu_S + J_R A_R \tag{2}$$

where $\Delta\mu_W$ and $\Delta\mu_S$ are, respectively, the chemical potential difference of water and solute.

By using non-equilibrium thermodynamics we can derive from Eqn 2 a set of phenomenological equations linearly relating the flows to the generalized forces through phenomenological coefficients L_{ik} describing the relation between the i-th flow to the k-th force. The matrix of phenomenological coefficients is supposed to be symmetrical, an assumption which has been questioned for membranes with chemical reactions [7] but which simplifies subsequent calculations without limiting their validity. We can make now the usual change of force and flow coordinates

$$\begin{aligned}
\Delta\mu_W &= \bar{V}_W \Delta P - \frac{\Delta\pi_S}{\bar{C}_W} \\
\Delta\mu_S &= \bar{V}_S \Delta P + \frac{\Delta\pi_S}{\bar{C}_S} \\
J_V &= J_W \bar{V}_W + J_S \bar{V}_S \\
J_D &= J_S/\bar{C}_S - J_W/\bar{C}_W
\end{aligned} \tag{3}$$

where $\Delta\pi_S$ is the partial osmotic pressure difference of solute S in the two compartments, $\bar{V}_i$ (i = W, S) represents partial molar volumes and $\bar{C}_i$ is the mean concentration of the solution components as defined by Kedem and Katchalsky [1]. Imposing the identity of Eqn 2 and of the new dissipation function containing flows and forces defined by Eqn 3, one obtains

$$\phi = J_V \Delta P + J_D \Delta\pi_S + J_R A_R \tag{4}$$

whence a new set of phenomenological equations can be derived, also bearing a symmetrical matrix of phenomenological coefficients. Of the new equations the one describing volumetric flow is

$$J_V = L_P \Delta P + L_{PD} \Delta\pi_S + L_{PR} A_R \tag{5}$$

where the phenomenological coefficients are given by

$$\begin{aligned}
L_P &= \bar{V}_W(L_{11}\bar{V}_W + L_{12}\bar{V}_S) + (L_{12}\bar{V}_W + L_{22}\bar{V}_S)/\bar{V}_S \\
L_{PD} &= \bar{V}_W(L_{12}/\bar{C}_S - L_{11}/\bar{C}_W) + \bar{V}_S(L_{22}/\bar{C}_S - L_{12}/\bar{C}_W) \\
L_{PR} &= L_{13}\bar{V}_W + L_{23}\bar{V}_S
\end{aligned}$$

and L_{ik} (i, k = 1, 2, 3) are the phenomenological coefficients relating the flows to the forces in the phenomenological equations associated to the dissipation function 2.

From Eqn 5 one obtains

$$\left(\frac{\Delta P}{\Delta\pi_S}\right)_{J_V = 0} = -\frac{L_{PD}}{L_P} - \frac{L_{PR}}{L_P}\frac{A_R}{\Delta\pi_S} \tag{6}$$

This equation shows that the ratio $(\Delta P/\Delta\pi_S)_{J_V = 0}$, employed by several authors as a measure of reflection coefficient [3, 4, 5, 8], is not a constant but depends upon $\Delta\pi_S$

and A_R, that is upon experimental conditions. Remembering that $\sigma = -L_{PD}/L_P$ and defining [2] $\sigma_{app} = (\Delta P/\Delta\pi_S)_{J_V=0}$, Eqn 6 becomes

$$\sigma_{app} = \sigma - \frac{L_{PR}}{L_P}\frac{A_R}{\Delta\pi_S} \tag{7}$$

which, if A_R is constant, represents a rectangular hyperbola in the plane $(\Delta\pi_S, \sigma_{app})$. The asymptote parallel to the $\Delta\pi_S$ axis intercepts the σ_{app} axis at the value σ.

Now, if experiments can be made in conditions where A_R is constant, Eqn 5 describes a plane in the space $(\Delta P, \Delta\pi_S, J_V)$. The intersection of this plane with the coordinate plane $J_V = 0$ is

$$-\Delta P = -\sigma\Delta\pi_S + L_{PR}\,A_R/L_P \tag{8}$$

and this equation, as well as the more general Eqn 5, can be used for σ determination as described in methods section. The same equation allows the determination of the apparent reflection coefficient and of its changes with experimental conditions.

As a consequence of previous calculations, the aims of the present work were: (a) The determination of $J_V(\Delta P, \Delta\pi_S)$ in conditions were it is likely that "active pump" works at saturation, so that A_R is constant, in order to check the validity range of Eqn 5. (b) The determination of the reflection coefficient for passive transport of a solute which is both actively and passively transported.

For such a study we used rabbit gall bladder because it possesses active transport of Na^+ and Cl^- in 1 : 1 ratio, without any important electrical potential difference [8, 9, 10] so that the salt reflection coefficient can be determined [11].

METHODS

Gall bladder preparation and flow measurements

Experiments were performed with everted gall bladders, taken from rabbits weighing about 2.5 kg, according to the technique described by Diamond [3]. Gall bladders with evident flogosis were discarded. The preparation was suspended in a 75 ml beaker containing about 50 ml of the perfusing solution and placed in a water bath at 25±0.1 °C. This temperature was chosen in order to have measurable fluxes and to avoid a rapid decay of the preparation. A mixture of 95 % O_2 and 5 % CO_2 was bubbled in the solution, taking care that the gall bladder was not touched by the bubbles.

The volumetric flow was measured gravimetrically, weighing the preparation every 4 min with a 10^{-4} g sensitivity balance. About 5 s after extracting the gall bladder from the perfusing solution the liquid drop under the preparation was dryed with filter paper without touching the organ. The operation was performed again 5 s later and weighing completed in about 20 s.

At the end of the experiment the organ surface was evaluated measuring its axes with a caliper and calculating the surface as a rotational ellipsoid. A second measurement was made cutting the gall bladder with a knife and distending the wall over a piece of graph paper. The two areas generally agreed within 10 % and the mean was assumed as the organ surface. Occasionally also the organ dry weight was measured, but flow normalization with this parameter has not been found useful.

Mounted gall bladders were filled with normal Krebs solution A until a slight distension occurred and perfused externally with the same solution for about 30 min before beginning the measurements. Subsequently the spontaneous volumetric flow was measured. This was 113.4 ± 18.2 mg/h or 28.4 ± 3.2 mg/h cm^2 or 7.30 ± 1.03 mg/h mg dry weight (means of 15 experiments in which both organ surface and dry weight were determined). After this first period we generally had 3–6 periods with external solutions generating opposite flows, in order to maintain the volume of solution A fairly constant inside the preparation, which was never changed during the whole experiment. Preliminary experiments showed that by such a procedure the osmolarity of the internal solution changed only a few percent with respect to the osmolarity difference between external and internal solution. Thus the flow alternation was needed only to maintain the organ volume to a constant value. Osmolarity measurements were made by the cryoscopic method. Subsequently the spontaneous volumetric flow was measured again, in order to have a check of the substrate characteristics constancy. If the two measurements of spontaneous J_V agreed within 15 % the experiment was continued with alternating flows, as before, with control periods every three to four measuring periods. The 15 % limit was chosen because such a variation induces a distortion in the fitting of data by means of a plane not bigger than that induced by the scatter of experimental points under the present conditions. As each flow determination generally requires 4 to 6 weighings, i.e. from 16 to 24 min, no more than 15 flows can be determined in a 4 h period, which seems to be the normal duration of our preparation with an acceptable constancy of characteristics, as determined from the constancy of volumetric flow during control periods and of the reflection coefficient at the beginning and end of the experiment. A weighing period of 16–24 min is almost always enough to reach a steady flow when the osmotic gradient is created on the mucosal side of the gall bladder, as observed also by Wright et al. [12].

Perfusing solutions

The composition of normal perfusing solution (type A) was: 118.4 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$. Other solutions were obtained by varying the NaCl content and adding sucrose at different concentrations in order to simulate the hydrostatic pressure difference by means of the osmotic pressure difference of an impermeant solute $\Delta\pi_i = -\Delta P$. The pH of the different solutions, buffered by means of 5 % CO_2 in oxygen, varied between 7.39 and 7.42. The osmolarity, ranging from 230 to 380 mosM, was calculated assuming the following osmotic coefficients [13]: NaCl 0.93, $NaHCO_3$ 0.96, KCl 0.92, $CaCl_2$ 0.86, KH_2PO_4 0.87, $MgSO_4$ 0.58, and sucrose 1.02.

The extreme osmolarity values were also measured cryoscopically. The agreement of calculated and measured values within the experimental error of a few percent, indicated that the above osmotic coefficients are valid in the concentration range of our solutions.

Reflection coefficient determination

The most probable values for the constants L_P, L_{PD} and $L_{PR}A_R$ of the plane defined by Eqn 5 in space (ΔP, $\Delta\pi_S$, J_V) were calculated by the least squares method together with their standard errors, utilizing the triplets of experimental data $\Delta\pi_i$,

$\Delta\pi_S$, J_V. $\Delta\pi_i$ and $\Delta\pi_S$ values were calculated using the above list of osmotic coefficients.

Regression analysis was performed by means of program BMD/03 R (Health Sciences Computing Facility, U.C.L.A.) adapted to the Univac 1106 computer of the University of Milan Computing Facility. The same program gives, by means of the Fisher Test, the probability that experimental triplets do not lay on a plane, here indicated as significance level p. The reflection coefficient was subsequently calculated from L_P and L_{PD} and its standard error evaluated from the standard errors of the phenomenological coefficients following the law of error propagation. The reflection coefficient can be calculated also my means of a graphical method, which does not allow standard error determination [14].

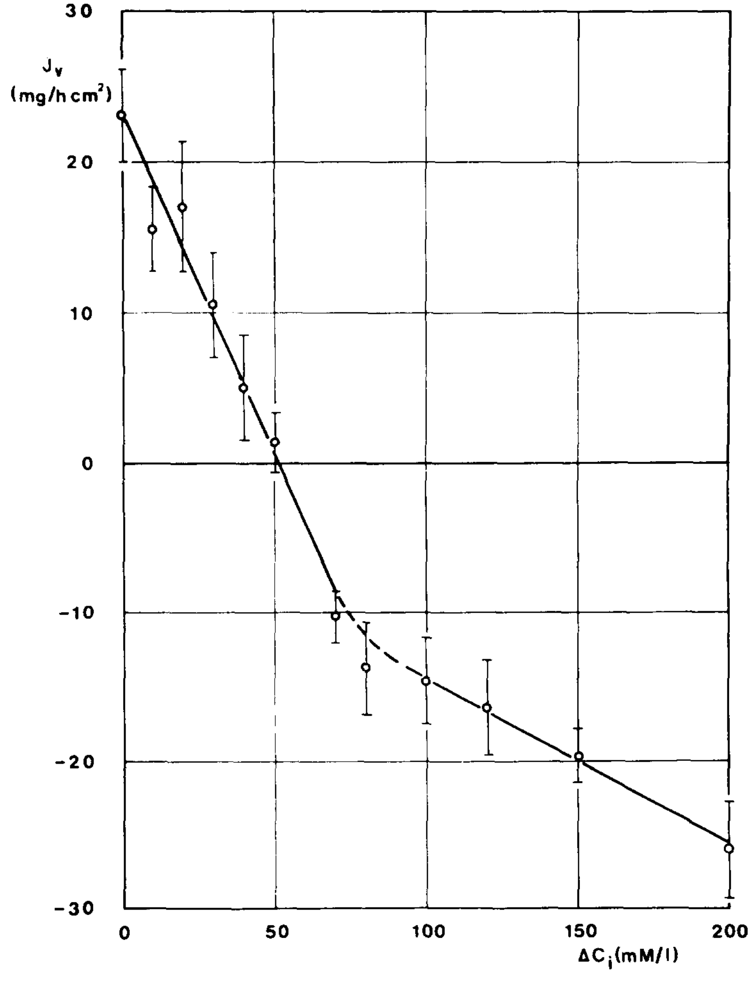

Fig. 1. Volumetric flow as a function of sucrose concentration difference.

RESULTS

Determination of linearity range

Linear non-equilibrium thermodynamics can be used only if all the flows are linearly related to the driving forces. Furthermore, as already pointed out in preceding sections, the active component of volumetric flow should remain constant when the composition of the perfusing solution is varied. Therefore, a preliminary investigation is required to define the range around physiological conditions where a linear dependence of volumetric flow upon the osmotic pressure differences can be assumed.

Experiments have been made with 15 gall bladders, varying sucrose concentration in a mucosal bathing solution between zero and 200 mM, while serosal solution type A remained unchanged. Sodium chloride concentration was therefore the same inside and outside the everted gall bladder. The results reported in Fig. 1 show that a linear dependence of J_V upon sucrose osmotic pressure difference can be assumed in the concentration range 0–80 mM. In this range the permeability coefficient L_P is $(0.507 \pm 0.058) \cdot 10^{-11}$ cm$^3 \cdot$ dine$^{-1} \cdot$ s^{-1}. For higher sucrose concentrations gall bladder permeability decreases to reach a new steady value at $(0.124 \pm 0.041) \cdot 10^{-11}$ cm$^3 \cdot$ dine$^{-1} \cdot$ s^{-1} above 100 mM sucrose.

The results of experiments with varying NaCl osmotic pressure difference are reported in Fig. 2. Negative $\Delta\pi_S$ values have been obtained by lowering NaCl concen-

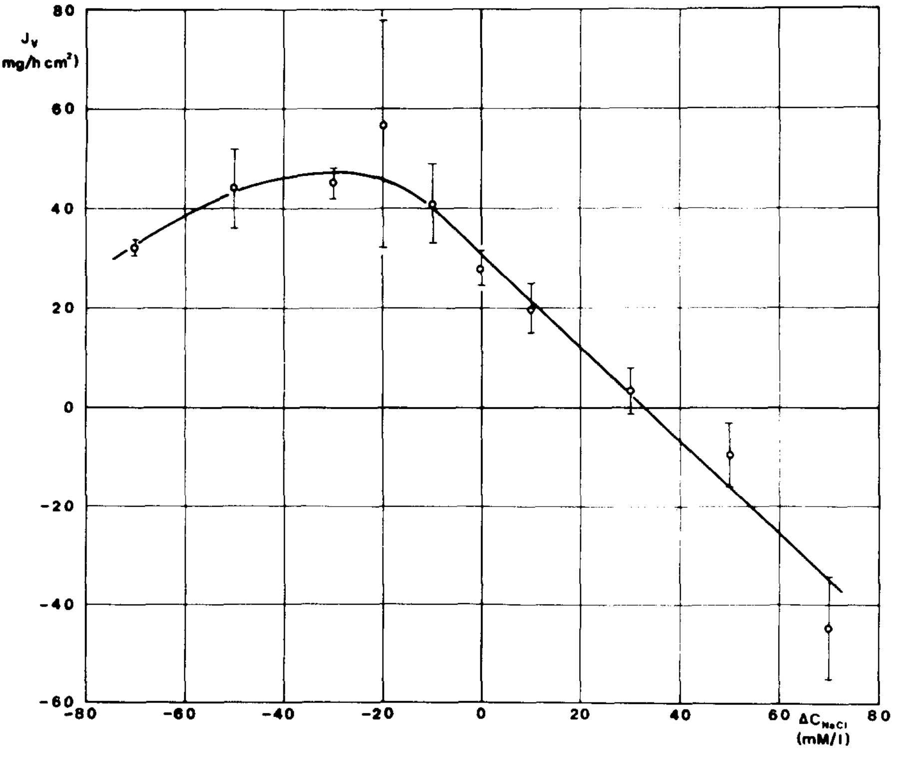

Fig. 2. Volumetric flow as a function of sodium chloride concentration difference.

tration in the outside solution, while positive $\Delta\pi_S$ values have been obtained by increasing the salt concentration in the same solution. A linear relationship between J_V and $\Delta\pi_S$ is obtained also in these experiments in the NaCl concentration range 20–70 mM.

Reflection coefficient determination

The volumetric flow values obtained at different sucrose and NaCl osmotic pressure differences in experiment no. 29 are collected in Table I. From these data and from twelve other similar experiments, it appears that volumetric flow is linearly related to $\Delta\pi_s$ and $\Delta\pi_i$. Thus the points determined by experimental coordinate triplets $\Delta\pi_i$, $\Delta\pi_S$ and J_V can be interpolated by a plane, as shown in Fig. 3 for experiment no. 32. The significance level of the plane is better than 1 % in 11 experiments and better than 5 % in other two experiments★.

The mean reflection coefficient, calculated from the data obtained in the above mentioned 13 experiments is 0.60±0.04.

TABLE I

Volumetric flows for various sucrose and sodium chloride concentration differences in one typical experiment. The reflection coefficient plus or minus the standard error and significance level p of the plane interpolating the experimental points are also given. Volumetric flow is positive when directed from serosa to mucosa and the concentration differences are positive when the concentration on the mucosal side is greater than on the serosal side.

Expt No.	ΔC_S (mM)	ΔC_i (mM)	J_V (mg/h)	$\sigma \pm$S.E.	p (%)
29	0	0	−96	0.55±0.06	1
	0	−80	138		
	10	0	−108		
	10	−80	138		
	0	−20	−30		
	−50	0	90		
	0	0	−102		
	10	−40	24		
	−10	−50	72		
	−10	0	−78		
	0	0	−108		
	−10	−25	6		
	−30	0	−6		

Apparent reflection coefficient

As shown by Eqn 7, the apparent reflection coefficient depends upon experimental conditions. For example, in experiment no. 32, reported in Fig. 3, it can be determined, by means of linear interpolation or extrapolation, that volumetric flow is zeroed by the following four ΔC_i and ΔC_S couples: −50, −10; −68, 0; −66, 10; and 0, 65 (mM). The ratios $\Delta\pi_i/\Delta\pi_S$ give the apparent reflection coefficients which are

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★ The values of the flows measured in all the 13 experiments can be obtained from G. Monticelli or F. Celentano.

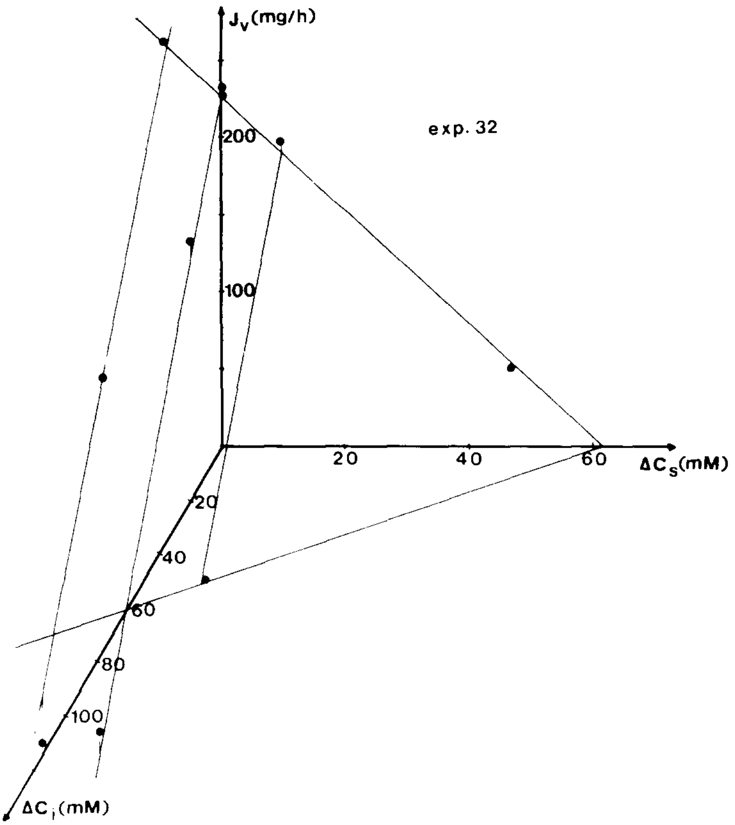

Fig. 3. Volumetric flow as a function of both sucrose and NaCl concentration differences in experiment no. 32. Experimental triplets of J_V, ΔC_S and ΔC_i are represented as points in the axonometric view and the distances from the interpolating plane are represented by vertical lines.

satisfactorily interpolated by the equation $\sigma_{app} = 0.51 - 33.1/\Delta C_S$ describing an hyperbola in the plane (ΔC_S, σ_{app}).

DISCUSSION

The reflection coefficient determination by our method requires the satisfaction of two fundamental assumptions, that is the constancy of substrate characteristics during the experiment and the linear dependence of flows upon their driving forces.

The first assumption has been verified checking the constancy of spontaneous volumetric flow, as described in methods section. Moreover σ values have been calculated in longer experiments with sets of seven points taken at the beginning (A), in the middle (B) and at the end (C) of the experiments. Mean σ values calculated with groups A, B and C in nine experiments $\bar{\sigma}_A = 0.59 \pm 0.06$, $\bar{\sigma}_B = 0.73 \pm 0.13$, $\bar{\sigma}_C = 0.57 \pm 0.05$ do not differ significantly with $\bar{\sigma} = 0.60 \pm 0.04$ calculated from all the thirteen gall bladders, indicating that all σ values are extracted from the same population. It follows that the constancy of gall bladder characteristics during the experiments is satisfactory.

As far as the linearity of flow-forces relationships is concerned, Figs 1 and 2 show that such a relationship can be assumed to exist up to 80 mM sucrose and 70 mM NaCl, but individual oscillations can be expected. We checked all the 13 experiments testing for any significant difference in the inclination of the semiplanes determined by interpolation of the points with high and low volumetric flow. The test was conducted as described by Williams [15] and no significant difference was found, indicating that all the points in each experiment can be interpolated by means of a single plane. In effect, as already mentioned, the probability that experimental points are not interpolated by a plane is less than 1 % in 11 experiments and less than 5 % in other two.

Non linear effects indeed occur, but outside the $\Delta\pi_i$ and $\Delta\pi_S$ ranges in which our experiments are made. The data plotted in Fig. 2 show that J_V decreases nonlinearly when the NaCl concentration in the mucosal solution is lowered below 90 mM, in agreement with the data by Diamond [16]. A disagreement with this author arises when NaCl concentration is increased above the physiological value, as we find a linear decrease of J_V with increasing NaCl concentration, while Diamond finds that J_V decreases nonlinearly and is never reversed. This behaviour is probably due to the technique employed by Diamond, who performed J_V measurements with the gall bladder suspended in air, without any serosal bathing solution. In such a situation there cannot be any fluid reabsorption. The nonlinear dependence of volume flow upon sucrose osmotic pressure difference above 80 mM sucrose concentration can be due to a modification of the epithelial structure, possibly a shrinking of the lateral intercellular spaces, as suggested by Diamond [17, 18].

The high standard errors of both permeability and reflection coefficients show that the selectivity and permeability of individual gall bladders are rather different, indicating a variable functional behaviour of the organ as a consequence of the complexity of its structure and of its relationships with the whole organism.

As has already been pointed out, the apparent reflection coefficient has no thermodynamic significance because it varies with the experimental conditions, as can be deduced from Eqn 7. Marro and Pesente [19] and Diamond [3] have calculated NaCl reflection coefficient values in frog skin and gall bladder as for a non-actively transporting membrane. These authors blocked the active transport eliminating NaCl on the epithelial (frog skin) or mucosal (gall bladder) side of the membrane. However the lack of the transported solute does not necessarily mean that the affinity of the chemical reaction, and thus active transport, is zero. In effect, zero volume flow can be given by equal and opposite passive and active components, a possibility which is accounted for in our treatment. Moreover, after NaCl withdrawal the membrane selectivity is probably different to that of the normally transporting membrane.

Thus it may be concluded that a measure of membrane selectivity for a species which is both actively and passively transported can be obtained only by accounting explicitly for the active transport contribution to volumetric flow, as in Eqn 5. This fact could explain the differences between the reflection coefficient values reported for rat kidney tubules [4, 5] and for rabbit gall bladder (ref. and the present work). In effect the σ value of 0.85 found by Diamond [3] is almost two standard deviations greater than our mean. Now, looking at the equation relating σ_{app} to ΔC_S in experiment no. 32, quoted in the preceding section, it appears that the σ_{app} value in Diamond's conditions should be about 0.2–0.3 higher than the true reflection coefficient.

Finally it should be noticed that a similar problem, i.e. the determination of the reflection coefficient for a passively transported solute in presence of active transport of another solute (urea in kidney tubules) has been previously solved, obtaining a linear dependence upon driving forces similar to Eqn 5 [20].

These considerations lead us to the conclusion that the gall bladder selectivity in physiological conditions can be measured describing the flows by means of the simplified model suggested by Kedem. However, the fit of Eqn 5 to experimental data is a necessary but not sufficient condition for the model validity, as it has been shown [21] that other descriptions lead to a linear equation $J_V = J_V(\Delta P, \Delta\pi_S)$ formally identical to Eqn 5. In any case it should be pointed out that in this model the gall bladder wall is a black box whose behaviour as a whole is considered. Thus the reflection coefficient measured by us is an index of the selectivity of the complex barrier, whose relationship with the selectivity of different membranes in series is not investigated.

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