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Ion-Mediated Water Flow*

II. Anomalous Osmosis

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Summary. Anomalous osmotic water flows may be the basis of the hypotonicity of gastric juice sampled at low rates of secretion. The anomalous osmotic flows of water produced by the exchange of hydrogen and a series of cations across the three membranes used in the electroosmotic studies (Paper I) have been obtained. The solvent flow results in part from the momentum imparted by the moving ions to the water contained in the membrane matrix. The physical parameters that regulate the rate of bi-ionic exchange and the accompanying anomalous osmotic solvent flows are: the hydration states of the membranes; the molarities of the membranes and the mobilities of the exchanging ions which are a function of ion size. Each ion in the exchange produces a flow of liquid. Assuming that the ions do not interact with one another in the membrane, the anomalous osmotic flux was assumed to be the sum of the water flows produced by each permeant ion. The anomalous osmotic flux produced by a bi-ionic exchange was calculated from electroosmotic coefficients ($[EO]_{\text{cation}}$ and $[EO]_{\text{Cl}}$) and the ion-exchange rates. The calculated values were all 10 to 60% less than the observed values. Part of these differences may have resulted from concentration gradients in unstirred boundary layers adjacent to the membrane which caused an osmotic flow of water in the direction of net water movement. As in the stomach, the sodium-hydrogen exchange produced a hypotonic solution.

Berkowitz and Janowitz [2] recently reported that when isoosmotic HCl is in contact with the resting gastric mucosa, H disappears and an almost

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equivalent amount of Na appears in the bathing solution. This exchange is accompanied by the flow of water from blood to lumen with the result that the bathing solution becomes hypotonic. These findings are at variance with previous experiments and theories [8, 9, 20, 21] as to the origin of the hypotonicity of gastric fluid observed at low volume rates of secretion [11, 12, 14]. In this communication a physico-chemical phenomenon which produces a flow of water against its gradient of chemical potential will be examined.

When two solutions of the same electrolyte at different concentrations are separated by a charged membrane, the flux of solvent that occurs may be far greater than that caused when nonelectrolytes are substituted for the electrolytes or the flux of solvent may be from the concentrated to the dilute solution [6, 7]. This phenomenon, which appears to defy the classical concepts of osmotic phenomena, has been called anomalous osmosis. In only a few instances has anomalous osmosis been studied with isoosmotic solutions of different electrolytes on either side of a membrane [7, 22], i.e., conditions of physiologic importance.

In the gastric instillation of isoosmotic HCl cited above [2], there was an almost 1:1 exchange of H for Na (H in the lumen exchanging for Na in the mucosa) and a flow of hypotonic solution into the pouch from blood to lumen resulted. The authors advanced the idea that an anomalous osmotic flux of solvent secondary to the diffusional exchange of H for Na might explain this observation. The solvent flow results in large part from the momentum imparted by the moving ions to the water in the cellular membrane [19, 22]. As the larger ion in the bi-ionic exchange has a lower mobility coefficient and consequently greater interaction with the solvent, net water flow will occur in the direction of exchange of the larger ion.

In the previous paper [16] current-induced flows of solvent (electroosmosis) were also shown to depend on ion size. The interpretation of electroosmotic solvent flows is simpler than that advanced to explain anomalous osmotic solvent flows since only a single counter-ion is involved in the first case [16]. However, the two phenomena, viz, an ion-mediated flow of solvent, share a common mechanism. The anomalous osmotic fluxes resulting from the exchange of H for Na, H for tetramethylammonium (TMA), and H for tetraethylammonium (TEA) across membranes of different hydrations and molarities were determined. The solvent flux by electroosmosis for each of these ions was determined in the same membranes and is reported in the previous paper [16]. If the same mechanism is responsible for water movement during current passage and bi-ionic exchange, then it should be possible to predict anomalous osmotic solvent flows using comparable electroosmotic data. The calculated anomalous osmotic fluxes were 10 to 60% less than those found experimentally. Unstirred boundary layers which induce osmotic water flows in the direction of net water movement during the bi-ionic exchange may be responsible for these differences.

Procedure

Fig. 1 is a schematic representation of the apparatus used to study bi-ionic exchange and anomalous osmosis across the three Biolon membranes (Amicon Corporation, Lexington, Mass.) used in the previous study [16]. The membrane was suspended between the two half-cells and the system made water-tight by an "*O*" ring seal. Solutions were pumped into each half-cell through a port at the base at 200 ml/min. Plastic netting in each half-cell helped to break up the laminar flow of the solution over the membrane surfaces. The rate of solution flow was constantly monitored. In all exchange experiments HCl solutions, 155 mEquiv/liter in concentration, were recirculated over the membrane on the "closed" side of the system which had a volume of 1,170 ml (*see* Fig. 1). The solutions containing the cations exchanging for hydrogen, Na, TMA or TEA, also 155 mEquiv/liter in concentration, were each passed over the membrane on the "open" side and were not recirculated. By this procedure the concentration of Na, TMA or TEA on the open side was constant and there was a negligible H ion concentration on this side. The gradients of both ions across the membrane were thus maximized and a complicated data analysis was avoided by preventing a back-exchange of H.

The amount of solvent moved across the membrane as a consequence of the bi-ionic exchange of H for Na, H for TMA, and H for TEA (the anomalous osmotic solvent flow) was obtained in the three membranes using the following procedure. The closed

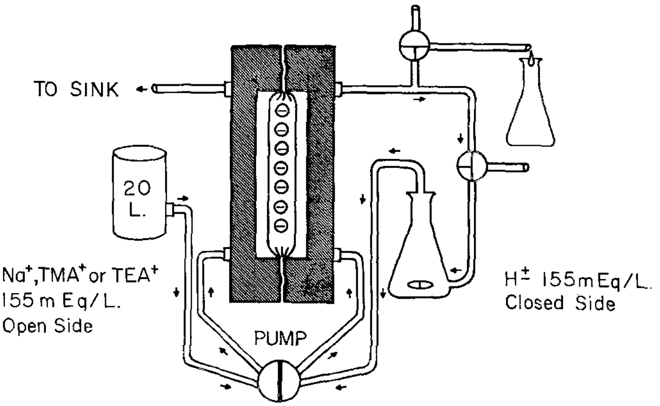

Fig. 1. Cell used to study bi-ionic exchange and anomalous osmosis. The membrane was secured between the two half-cells (constructed of polymethylmethacrylate) by an "*O*" ring gasket. The solution entering the closed side as a result of bi-ionic exchange flowed out of the side-arm and was collected in tared vessels. Ion-exchange rates were determined by replacing the closed erlenmeyer with an open beaker from which aliquots of solution for assay could be withdrawn. The same volume of HCl solution (1,170 ml) was used in both anomalous osmosis and ion flux determinations

side of the system was filled to the upper stopcock and side-arm. As fluid entered the closed side as a result of the exchange, the increase in volume caused the fluid to flow out of the side-arm and it was collected in tared vessels over 5-min time intervals. The amount of solution transported was determined gravimetrically. Sixteen collection periods comprised a run. At least two separate determinations of anomalous osmotic solution flows were made for each exchanging pair of ions. The duplicate runs were not made consecutively.

The change in the ionic composition of the acid side was determined as the exchange proceeded in a parallel set of experiments. The same experimental conditions were employed in both types of experiments to quantitatively relate ion exchange and its resultant solvent flow. The closed erlenmeyer shown on the right side of Fig. 1 was replaced by an open beaker and the stopcock and side-arm removed. The same volume of acid (1,170 ml) was added to the open beaker as was contained in the closed system. Aliquots of 5-ml each were withdrawn from the beaker at 15-min intervals and the samples assayed for H-ion by titration with standardized NaOH and chloride ion with an Aminco-Cotlove Automatic Chloride Titrator. Osmolality was determined by freezing point depression (Advanced Instruments, Model 31WAS). The exchange of each of the three ions with H was performed two to five times for each membrane. Repetitive runs were not made consecutively.

Anomalous osmotic solvent flows and ion fluxes for *only* the Na-H exchanges were also determined in a fourth membrane (IV) of low water content and high permselectivity. The membrane [1], composed of 35% polystyrene sulfonic acid (PSSA), 10% Epon (epoxy, Shell Chemical Corp.), and 55% Kynar (polyvinylidene fluoride, Pennsalt Chemical Corp.), was 25% hydrated in the H form and its cation transport number was 0.99 in a solution of KCl (concentration potential measurements, 0.10 M KCl *vs.* 0.20 M KCl).

Results

The physical properties of the three Biolon membranes-their ion exchange capacities, molarities, thicknesses and water contents are shown in Table 1 in the previous paper [16] and their permselectivities in Table 2 [16].

Fig. 2 presents the rates of solution flow resulting from the bi-ionic exchange of Na and H across the PSSA-Kynar membrane (IV) and the three Biolon membranes. With time, the amount of solution collected declined. The other two bi-ionic exchange systems (TMA-H and TEA-H), which were only studied in the three Biolon membranes, displayed the same solution flux behavior. Inconsistent volume flows occurred in the first four collection periods (*see* Discussion). The remaining 12 collection periods (25 to 80 min) fit an equation of the form:

$$w = A_0 e^{Bt} \tag{1}$$

where A_0 is the instantaneous solution flow at zero-time, w the solution flow at time t, and B the rate of change of solution flow. The values for the constants A_0 and B (Table 1) were obtained by the usual least-squares procedures. For all exchange systems, the rate of solution flow into the

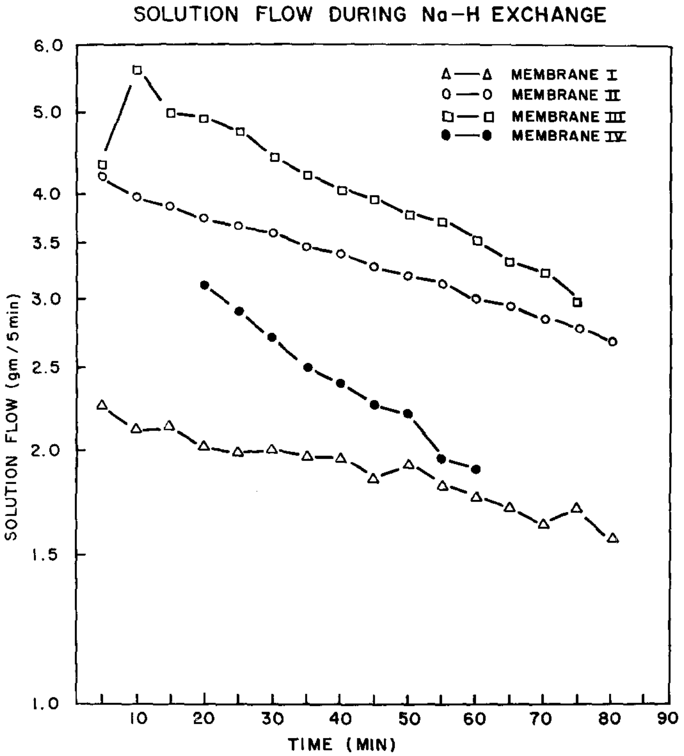

Fig. 2. The anomalous osmotic solution flows resulting from Na-H exchange across three Biolon membranes (I, II, and III) and one membrane made of polystyrene-sulfonic acid and Kynar (IV). I (Δ—Δ) was 43% hydrated; II (○—○) 60% hydrated; III (□—□) 58% hydrated; and IV (•—•) 25% hydrated

Table 1. A_0 and B values calculated from Eq. (1)

Membrane	Bi-ionic exchange system Na-H		TMA-H		TEA-H	
	A_0 (g)	B (10^5/sec)	A_0 (g)	B (10^5/sec)	A_0 (g)	B (10^5/sec)
I	2.11	− 6.33	2.09	− 2.81	1.76	−1.94
II	4.18	− 9.41	4.50	− 6.52	3.73	−3.76
III	5.74	−14.57	6.26	−11.16	5.70	−7.73

closed side increased as the hydration and molarities of the membranes increased (parameter B, Table 1). However, the flow rate declined in any membrane as the hydrated radius [15] of the cation exchanging for hydrogen became larger.

Table 2. Decline in solute concentration on acid side during Na-H exchange

Membrane IV					Membrane I				
Time (min)	Cl^- (mEquiv/liter)		Osmolality (mOsm/kg H_2O)		Time (min)	Cl^- (mEquiv/liter)		Osmolality (mOsm/kg H_2O)	
	cal[a]	obs[b]	cal	obs		cal	obs	cal	obs
0	–	162	–	312	0	–	155	–	297
5	161	163	311	311	15	154	154	295	293
20	160	160	308	307	30	153	152	293	290
40	158	160	305	304	45	153	150	291	286
60	157	158	303	302	60	152	148	290	283
90	156	157	300	299	75	151	147	289	280

[a] cal are calculated values.

[b] obs are observed values.

Table 3. Zero-time ion and water fluxes for the bi-ionic exchanges of Na-H, TMA-H and TEA-H

System	Fluxes (Zero-time) $(mmoles/sec/cm^2) \times 10^5$			
	H^+	X^+	Cl^-	H_2O
Membrane I: 43 % hydrated; molarity 0.42 $mEquiv/cm^3$				
Na-H	−6.29	5.81	−0.49	147
TMA-H	−4.80	4.41	−0.40	122
TEA-H	−3.57	2.55	−0.95	100
Membrane II: 60 % hydrated; molarity 0.43 $mEquiv/cm^3$				
Na-H	−7.30	6.43	−0.90	276
TMA-H	−5.64	4.58	−1.06	279
TEA-H	−4.38	3.00	−1.48	229
Membrane III: 57 % hydrated; molarity 0.58 $mEquiv/cm^3$				
Na-H	−9.96	8.84	−1.03	379
TMA-H	−8.57	7.43	−1.15	378
TEA-H	−6.40	4.69	−1.94	347

Negative sign indicates the ion is leaving the acid side. X^+ represents the cation exchanging for H^+.

The ion exchange rates were determined by assaying the acid side for H and Cl ion concentration at various time intervals during bi-ionic exchange. Fluid entered the acid side as a result of the bi-ionic exchange causing a change in volume. In the calculation of the net ionic changes the increase in volume produced by the anomalous osmotic flux of solvent [*see* Eq. (1)] during a given time period was taken into consideration. For the Biolon I membrane and the PSSA-Kynar membrane (IV), the decline in Cl concentration and osmolality on the acid side of the system during Na-H exchange is shown (Table 2). The observed decline in these measures of solute concentration is compared to the fall expected from the entry of solvent into the acid side. In membrane IV there is no difference ($p > 0.05$) between the calculated and observed decline in solute concentration. For membrane I, the observed decline in solute concentration exceeded the fall expected from the flow of solvent into the acid side of the system. The calculated values for all ionic changes, H, Na, TMA, TEA and Cl were plotted against time and extrapolated to zero-time. The rates and direction of ion movement at zero-time for all exchanging ions are listed in Table 3. The rate of cation exchange increased as the hydration and the ion-exchange capacities of the membranes increased. In any membrane, however, as the hydrated radius of the ion exchanging for hydrogen became larger, the rate of cation bi-ionic exchange declined and the rate of chloride loss from the acid side increased.

The net amount of solution entering the closed side at zero-time as a result of the bi-ionic exchange is given in grams (parameter A_0, Table 1). The solution flow rates listed on the left side of Table 4 were determined by taking into account the membrane area (268 cm^2) and the time interval for solution collection (300 sec). The molar solvent flows (right side of Table 4) were calculated from the solution flow rates by the equation:

$$(\Delta H_2O) \times 18 = \Delta(\text{solution}_{A.O.}) - \bar{V}_{\text{cation}}(C_{0,\text{cation}}) + \bar{V}_{H}(C_{0,H}) \tag{2}$$

where Δ ($\text{solution}_{A.O.}$) is the rate of solution transport determined experimentally, $\bar{V}_{\text{cation}}$ and $\bar{V}_H$ the partial molar volumes of the larger cation and hydrogen, respectively, and C_0 the zero-time ion exchange flows (Table 3). Correcting the solution flow for the Na-H exchange leads to almost no change reflecting the similar molar volumes of NaCl and HCl (center section,

Table 4. Anomalous osmotic flux during bi-ionic exchange

System	Solution rate (g solution/sec/cm^2) × 10^5			Water rate[a] (g water/sec/cm^2) × 10^5			(mmoles H_2O/sec/cm^2) × 10^5		
	Membrane			Membrane			Membrane		
	I	II	III	I	II	III	I	II	III
Na-H	2.62	5.19	7.13	2.64	5.21	7.16	147	289	398
TMA-H	2.60	5.59	7.78	2.20	5.17	7.09	122	287	393
TEA-H	2.19	4.64	7.09	1.81	4.20	6.40	100	233	355

[a] Corrections were calculated from solution rates using Eq. (2).

Table 4). Substantial changes occur however when the TMA and TEA anomalous osmotic fluxes are treated in this fashion. The rate of solvent flow was converted from g/cm^2 per sec to moles/cm^2 per sec by using the molar volume of water, 18 g/mole. For all three exchange systems, greater amounts of solvent flowed as the hydration of the membranes and their molarities increased.

Discussion

It has been demonstrated that there is a transport of water from blood to a solution of lower tonicity in the stomach. Lifson, Varco and Visscher [11] and others [12, 14] have found that at low volume rates of gastric secretion the gastric juice becomes hypotonic with respect to plasma. The principal cation in this secretion is sodium [14]. In recent experiments, Berkowitz and Janowitz [2] instilled isoosmotic HCl solutions containing a nonabsorbable dilution indicator into vagally denervated fundic pouches in unanesthetized dogs. The pouch was a closed system from which complete recovery of solution was possible and this was verified by 95% recoveries of the dilution indicator. Significant errors in recovery were further minimized by the large volumes, 40 to 70 ml, employed. They [2] found that solute concentration fell as the Na-H exchange proceeded and this was accompanied by an increase in fluid volume. Furthermore, there was a net increase in solute as manifest by an increase in total chloride in the luminal fluid. The increase in chloride correlated almost quantitatively with the difference between the sodium gain and hydrogen loss from the pouch. The concentration of solute transported in the fluid moved from mucosa to lumen was about 150 mOsm/kg H_2O [2]. It thus appears that the decline in solute concentration observed in many recent experiments [3–5, 13] involving the instillation of acid into the resting stomach is the result of the flow of a hypotonic solution into the stomach. The basis of this hypotonic flow of solution may be the bi-ionic exchange of Na for H.

The experiments involving the exchange of Na for H across ion-exchange membranes indicate that a bi-ionic exchange can produce a flow of hypotonic fluid to the acid side of the system (Table 2). The PSSA-Kynar membrane is perfectly cation selective and it is not surprising that the anomalous osmotic flux of water accounted for the decline in solute concentration on the acid side (Table 2). In the case of the Biolon I membrane, the observed decline in solute concentration exceeded the decline expected from the anomalous osmotic flux of fluid (Table 2). The Biolon membrane was not perfectly cation selective and small amounts of chloride left the acid side of this system. The observed decline in solute concentration was the result of the anomalous osmotic flux of fluid and the chloride transport out of the acid side. Approximately half of the decline in solute concentration for membrane I during Na-H exchange was caused by the anomalous osmotic flow of water and the remainder was the result of chloride transport out of the acid side.

These synthetic membrane experiments have permitted the development of a molecular model for the transport of water against its gradient from blood to gastric lumen in the cited physiologic experiments. As a result of the synthetic membrane experiments several of the physical parameters that regulate the rate of bi-ionic exchange and the accompanying anomalous osmotic solvent flows have been delineated. Two are intrinsic to the membrane – the hydration state and the number of charged groups. A third important parameter, the mobilities of the exchanging ions, is a function of the size of the hydrated ions. These factors are summarized in the Nernst-Planck flux equation:

$$\bar{J}_i = -\bar{D}[\bar{C}_i(d\ln\bar{a}_i/dx) + Z_i(F/RT)\bar{C}_i(d\phi/dx)] + \bar{C}_i v^* \qquad (3)$$

where $\bar{J}_i$ is the flux of species i through the membrane. The net flux $\bar{J}_i$ is the result of a number of separate fluxes. Two of these fluxes are contained in the brackets. The first, due to diffusion, results from concentration gradients and is given by $\bar{D}\bar{C}_i(d\ln\bar{a}_i/dx)$, where $\bar{C}_i$ is the concentration and $\bar{a}_i$ is the activity of species i in the membrane. The second term represents the flux of species i caused by an electric field $(d\phi/dx)$ generated when ions of different mobilities exchange for one another across a charged membrane. The last term in Eq. (3), $\bar{C}_i v^*$, the convection term, is often neglected in considering ion-exchange phenomena in classical thermodynamic or quasi-thermodynamic treatments. It is a term distinctly unique to ion-exchange membranes as will be shown.

When an electric field acts on an electrolyte solution, the ions move toward their respective electrodes and except for solvation shells there is no transfer of solvent. A net transfer of solvent can be observed however when a charged membrane is placed between the two electrodes (an electro-osmotic transfer of solvent to be precise). This occurs because the membrane is permselective and contains more counter-ions than co-ions. The counter-ions are in the majority and impart more momentum to the solvent in the membrane matrix than do the co-ions. With the imposition of an emf, the net result is a unidirectional flow of solvent in the direction of the counter-ions. The rate v^* at which (the center of gravity of) the pore liquid moves is determined by the strength of the electric field which drives the ions and the interactions between the ions and the pore liquid.

In the bi-ionic exchange an external field is *not* applied but rather an electrical-potential gradient set up by the exchanging ions acts as an equivalent driving force. As in the electroosmotic case, the membrane phase permits counter-ions to pass and retards co-ions. In the bi-ionic system however, the direction of net solvent flow is determined by the amount of solvent transported by each counter-ion[1]. The counter-ion which imparts the greatest momentum to the surrounding medium will cause net solvent flow to occur in its direction of exchange [22]. Consequently, in any membrane, it is the ion of lowest mobility that will determine the direction of net solvent flow during bi-ionic exchange. The mobility of an ion in solution is related to its radius [18]. The larger the ion the greater will be its interaction with the surrounding medium and the lower its mobility. For the ions used in this study, the TEA ion had the lowest equivalent conductivity (32.7 cm^2/Ω per Equiv), followed by TMA (44.9 cm^2/Ω per Equiv), Na (50.1 cm^2/Ω per Equiv), and finally H (349.8 cm^2/Ω per Equiv) [18]. Net solvent flow for the three exchange systems, Na-H, TMA-H, and TEA-H was in the direction of flow of the cation exchanging for H, behavior predicted by the above considerations.

The anomalous osmotic flux obtained experimentally (Table 3) is a net solvent flow with each ion involved in the exchange causing a flow of liquid. If it is assumed that the ions as they move through the membrane do not interact with each other, the resulting solvent flow is composed of the following independent fluxes:

$$J^{w}_{\mathrm{A.O.}}(\exp) = J^{w}_{(\mathrm{C})} - J^{w}_{(\mathrm{H})} - J^{w}_{(\mathrm{Cl})}. \tag{4}$$

Each J^w is the solvent flow in moles/sec accompanying H, Cl or one of the cations exchanging for hydrogen. The fluxes associated with the movement of H and Cl are subtracted as they remove solvent from the measuring chamber. The solvent flow associated with individual ion movement (J^w) cannot be determined experimentally in anomalous osmosis. If however, in anomalous osmosis and electroosmosis, the same mechanism is responsible for solvent transport, the solvent flow associated with individual ion movement can be calculated from the electroosmotic coefficient (in moles H_2O/Equiv) and the rate of ion movement (in Equiv/sec) obtained in bi-ionic

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[1] An osmotic flow of water can result from unstirred boundary layers adjacent to the membrane and contribute to the observed water flow. These comments apply explicitly however when perfect stirring can be maintained to the membrane-solution interface. We will come back to this problem.

exchange experiments. Eq. (4) can then be rewritten as

$$J^{w}_{\mathrm{A.O.}}(\mathrm{calc}) = J^{w}_{\mathrm{E.O.(C)}}(Eq^{0}_{C}) - J^{w}_{\mathrm{E.O.(H)}}(Eq^{0}_{\mathrm{H}}) - J^{w}_{\mathrm{E.O.(Cl)}}(Eq^{0}_{\mathrm{Cl}}) \tag{5}$$

where the $J^{w}_{\mathrm{E.O.}}$ are the electroosmotic flows of solvent for the respective ions and the Eq^{0} are the zero-time ion exchange values.

Eq. (5) has been written on the assumption that the same physical phenomenon is responsible for solvent flow in electroosmosis and anomalous osmosis. It has been argued that in both processes water movement results from the momentum transferred by a moving ion. In electroosmosis, an externally applied electric field is the force that drives the ions through the charged membrane; in anomalous osmosis, the flux of ions results from an electrochemical potential across the permselective membrane. However, the forces responsible for driving the ions in the two methods need not be the same. It is only necessary that the physical process involve a transfer of momentum from the ions to the medium. It seems likely that in both anomalous osmosis and electroosmosis such a transfer occurs and it may be assumed then that the amount of solvent moved by an ion during bi-ionic exchange is the same as that moved by the ion during electroosmosis.

In any membrane the solvent flow ($J^{w}_{\mathrm{A.O.}}$) remained the same or declined as the size of the ion exchanging for H increased (Table 3). The reasons for the decline can be understood at least partly in terms of Eq. (5). It is clear from Eq. (5) that the number of ions diffusing across the membrane and the amount of solvent transferred by the ions are both important in determining solvent flow. The amount of solvent each ion transported increased as the hydrated radii of the ions increased (Table 5, previous paper [16]). However, as the size of the ions exchanging for H increased, the rate of bi-ionic exchange declined (Table 3). The rate of exchange of TMA and TEA (for H) was much less than the Na rate in a given membrane. However, the observed anomalous osmotic flows with these ions were about equal to that of the Na. It is obvious then that the larger ions must move more water per mole than Na. The importance of the Cl ion diffusion on $J^{w}_{\mathrm{A.O.}}$ (exp) is shown by Eq. (4). In all three membranes, Cl losses from the acid side were 70 to 100% greater in the TEA-H system than in the Na-H system (Table 3). The movement of Cl ion produces a solvent flow opposite to that produced by the larger cation. The effect of increased Cl diffusion is to reduce further the observed anomalous osmotic flow which is produced primarily by the flux of the larger cation.

Membranes I and II contain almost the same amount of ionized sulfonic acid groups, but membrane II contains 17% more water. The identical number of anionic groups contained in the two membranes is reflected in the similar bi-ionic exchange rates, e.g., differences of only 20% in column X^+ (Table 3). However, there is a significant effect of membrane water content on the amount of solvent moved across each membrane (Table 3, last column). In the more hydrated membrane (II) the solvent fluxes increased by at least 100% over those found for the same exchange systems in the less hydrated membrane (I). The increased $J^{w}_{\mathrm{A.o.}}$ (exp) in the more hydrated membrane results in part from the larger quantities of solvent transported by the ions. The corrected electroosmotic coefficients ($J^{w}_{\mathrm{E.o}}$) for the cations in membrane II exceeded those found for membrane I by 90 to 130% (Table 5, [16]).

The effects of different numbers of ionized groups in membranes that contain the same amounts of water can be seen in the anomalous osmotic fluxes obtained in membranes II and III (Table 3). Membrane III contained 40% more anionic groups than membrane II. The rates of ion exchange were larger in the membrane of greater molarity – 30 to 50% greater than in membrane II. The increased solvent flows in membrane III may only reflect the increased cation fluxes which were of the same order of magnitude.

Using Eq. (5), anomalous osmotic solvent flows for the three bi-ionic exchange systems were calculated from electroosmotic and ion flux data (Table 5). The calculated values were all 10 to 60% less than those found experimentally. Some of the differences between the calculated and observed anomalous osmotic values may have resulted from variations in the internal environment of the membrane These include: (1) The membranes were in different physical states during electroosmosis and anomalous osmosis. During electroosmosis the membrane contained a single cation at a fixed concentration and a given amount of solvent. The same membrane during anomalous osmosis contained two cations, hydrogen and one of the larger ions, and a different amount of solvent than when the membrane was in a solution of one or the other of the two cations. (2) At the beginning of each bi-ionic exchange the membrane was in the H form. At zero-time,

Table 5. Comparison of anomalous osmotic fluxes determined experimentally and calulated from Eq. (4)

System	Water rate (mmoles $H_2O/sec/cm^2$) × 10^5					
	Membrane I		Membrane II		Membrane III	
	Exp	Cal	Exp	Cal	Exp	Cal
Na-H	146	89	289	145	398	229
TMA-H	122	83	287	171	393	361
TEA-H	100	57	233	87	355	211

the cation exchanging for H was circulated over one side and began to diffuse into the membrane. Until an equilibrium distribution of the two cations had occurred however, a nonsteady-state condition existed in the membrane and may have caused at least partially the erratic flows observed during the first 20 min of bi-ionic exchange.

Unstirred boundary layers, which could have caused an osmotic flow of water in the direction of flow of the larger ion may have played an even larger role than the factors cited above in causing the differences between the observed and calculated anomalous osmotic solvent flows. During anomalous osmosis, in contrast to electroosmosis, solution was constantly passed over the membrane faces at a rate of 200 ml/min. At this rate an unstirred boundary layer of approximately 160 μ was present[2] and local concentration gradients may have developed. In the previous paper [16] the same phenomenon was observed during current passage and was attributed to differences in ion transport numbers between the membrane and bulk solutions. During electroosmosis the accumulation or depletion of solute in the unstirred layers that resulted from these ion transport number differences caused an additional osmotic flow of water in the direction of current passage. The magnitude of the water flow was estimated and found to represent from 10 to 40% of the total current-induced water flow.

With the experimental techniques employed in the anomalous osmosis experiments, the contribution of osmotic water flow (resulting from unstirred boundary layer effects) to the net water flow during bi-ionic exchange cannot be directly evaluated. An estimate of the maximum osmotic contribution can be made by using the osmotically induced water flow rates found during electroosmosis (Table 3, previous paper [16]). In brief, the calculation depends on comparing the ion flux during anomalous osmosis (Table 3) to the equivalent ion flux produced during current passage in electroosmosis. Assuming a linear relationship between these rates, a comparable water flow rate for each cation during bi-ionic exchange was calculated and the values listed in Table 6. It was assumed that no osmotic flow of water occurred in the direction of H flow during bi-ionic exchange as current passage did not produce an osmotic flow of water in solutions of HCl. For this reason the osmotic water flow rates listed in column A, Table 6 were calculated only for the cations Na, TMA and TEA.

In column B, Table 6, the differences between the experimentally obtained anomalous osmotic water flows and the calculated values are listed. It is apparent that between 40 and 100% of these differences may have resulted

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[2] J.M. Berkowitz, *unpublished data.*

Table 6. Osmotic water flow rates during bi-ionic exchange

System	Water rate (mmoles $H_2O/sec/cm^2) \times 10^5$					
	Membrane I		Membrane II		Membrane III	
	A	B	A	B	A	B
Na-H	23.0	57	48.0	144	48.9	169
TMA-H	50.3	39	51.2	116	54.1	32
TEA-H	36.5	43	59.0	146	56.0	144

A. Calculated osmotic water flow in direction of net water flow during bi-ionic exchange.

B. Difference between the experimentally obtained anomalous osmotic water flow and the calculated value (Table 5).

from osmotically induced water flows. This estimate must be treated with some caution, however, as rapid stirring was maintained during bi-ionic exchange but there was no stirring during current-induced solution flow. As the extent of the unstirred boundary layer is reduced by rapid stirring [10] there is little doubt that the stagnant films were more extensive in the latter case. Nevertheless, a substantial contribution to the total water flow during bi-ionic exchange may have resulted from an osmotic flow of water caused by solute concentration changes in the unstirred boundary layers adjacent to the membrane.

It has long been recognized that unstirred boundary layers may play an important role in controlling ion and water movements in synthetic and biological membranes. Of particular relevance to the present case is the mechanism of transcellular water transport in mammalian gastric mucosa proposed by Rehm, Butler, Spangler and Sanders [17]. Using a conceptual model, they postulated the development of a hypertonic solution in the gastric pits during Na-H exchange across a flat membrane at its base followed by an osmotic flow of water from blood to gastric lumen. In this proposal the hypertonic solution that develops is the result of unstirred boundary layers adjacent to the cellular membrane. Rehm and his colleagues have neglected the issue raised in this communication, viz, the ion-mediated movement of water across ion-exchange membranes. However, there is no reason to assume only one mechanism is operative in producing the observed hypotonic flow. Indeed, based on the morphological characteristics of the gastric pits, it seems more likely that an ion-mediated water flow resulting from a bi-ionic exchange of Na and H and an osmotically induced water flow caused by unstirred boundary layers would occur.

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