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EFFECTS OF ELECTRICAL GRADIENTS ON VOLUME FLOWS ACROSS GALL BLADDER EPITHELIUM

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SUMMARY

A volumetric method has been developed which permits continuous registration of volume flows across epithelial tissues. The method was applied to volume flow measurements across rabbit gall bladder epithelium. The rate of fluid reabsorption measured in this way was twice as high as previously observed in sac preparations of the gall bladder. This is probably due to better aeration and stirring of the mucosal solution. It was demonstrated that electrical gradients across the gall bladder induced volume flows towards the negative electrode. In non-transporting bladders volume flows were linearly related with current between 300 and 900 μA in both directions. However, volume flow rates were three times higher from mucosa to serosa than in the opposite direction. From the magnitude of polarization potentials, observed after switching off the current, the conclusion was reached that all of the current-induced volume flow is an osmotic flow due to salt polarization in the unstirred layers of the tissue. By implication, so-called streaming potentials observed during osmotic flows reflect solely polarization effects. In actively transporting gall bladders a 200 μA current increased or decreased the flow rate twice as much as expected from polarization effects alone. Therefore passage of current interfered directly with the active transport mechanism of gall bladder epithelium.

INTRODUCTION

In studying volume flows across gall bladder epithelium gravimetric methods have been used thus far [1, 2]. In essence, they consisted in weighing gall bladder sacs at intervals of at least 5 min. Disadvantages of this approach are a poor time resolution and that the solution on one side of the epithelial cells is essentially unstirred. The importance of a good time resolution in flow measurements is stressed by Wright et al. [3] who showed that flow rates drop to one-fifth of the initial rate within 5 min of applying an osmotic gradient to the mucosal side of the epithelium. Good stirring of mucosal solutions is equally important as has been shown by Bindslev et al. [4].

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These authors observed that frog gall bladders, mounted as flat sheets between lucite half chambers, developed spontaneous transmural potentials under good stirring conditions, while switching off stirring caused the potentials to fall to near zero.

In sac preparations of gall bladders it has been shown that passage of current induced water flow towards the negative electrode [5]. However, a flat preparation would be more desirable in studying effects of electrical gradients, since the electric field will be more homogeneous.

For these reasons we have developed a volumetric method which permits continuous registration of water flow across flat epithelial tissues. This paper reports application of the volumetric method while studying effects of electrical gradients across non-transporting and actively transporting gall bladders.

METHODS AND MATERIALS

Volume flow measurement

Rabbit gall bladders were prepared as described previously [6]. Ringer solutions of the following composition were used (mM): NaCl, 125; $NaHCO_3$, 25; KCl, 3.8; $CaCl_2$, 1.0; $MgCl_2$, 1.0; KH_2PO_4, 1.2; pH, 7.4. Solutions were gassed with water-saturated gas mixtures (95 % O_2/5 % CO_2). Isolated gall bladders were cut open and mounted as a flat sheet between two lucite half-chambers, designed as shown in Fig. 1. Leakproof seals were ensured by rubber O-rings. The areas of the opening between the two halves was 0.78 cm^2 and each reservoir contained approximately 3 ml Ringer solution. The solution in the mucosal reservoir was recirculated by means of an oxygen gas lift. The temperature of the circulating solution was thermostatically controlled such that the temperature of the mucosal solution was 38 ± 0.5 °C. The serosal solution, the volume of which is monitored, was not thermostatically controlled. Since the epithelial cells on the mucosal side constitute only 10% of the thickness of the bladder wall, the temperature gradient across the epithelial cells is negligible. A glass capillary (0.5 mm internal diameter) was mounted vertically on the serosal compartment. The fluid level in the capillary was monitored by a photocell, the output of which was connected to a relay (Visolux-Electronik, Type ML). The relay was also connected to an electric motor which drove a microburette mounted into the serosal compartment (Fig. 1). Volume changes in the serosal compartment were monitored by the photocell, and compensated by forward or backward movement of the motor-driven microburette. The displacement of the microburette was measured by means of a displacement transducer (Hewlett-Packard 7 DCDT-1000). The output of the transducer was connected to one channel of a two-channel pen recorder (Servogor RE 520). A block diagram of the automatic volume registration is shown in Fig. 2. In this system a 1-mV change in the output voltage of the transducer corresponded to 0.206 μl. The minimal volume change detected by the photocell and compensated by means of the microburette was about 0.05 μl. A typical example of an automatic volume registration is shown in Fig. 3. In order to measure volume changes in the serosal compartment a small mucosal positive pressure (of about 2 cm H_2O) is required to keep the tissue pressed against an appropriate support (nylon mesh with pores of 0.2 mm). During flow measurements both compartments were stirred by means of teflon-coated magnetic stirrers which were inserted through the chamber wall and were each rotated by a motor-driven external

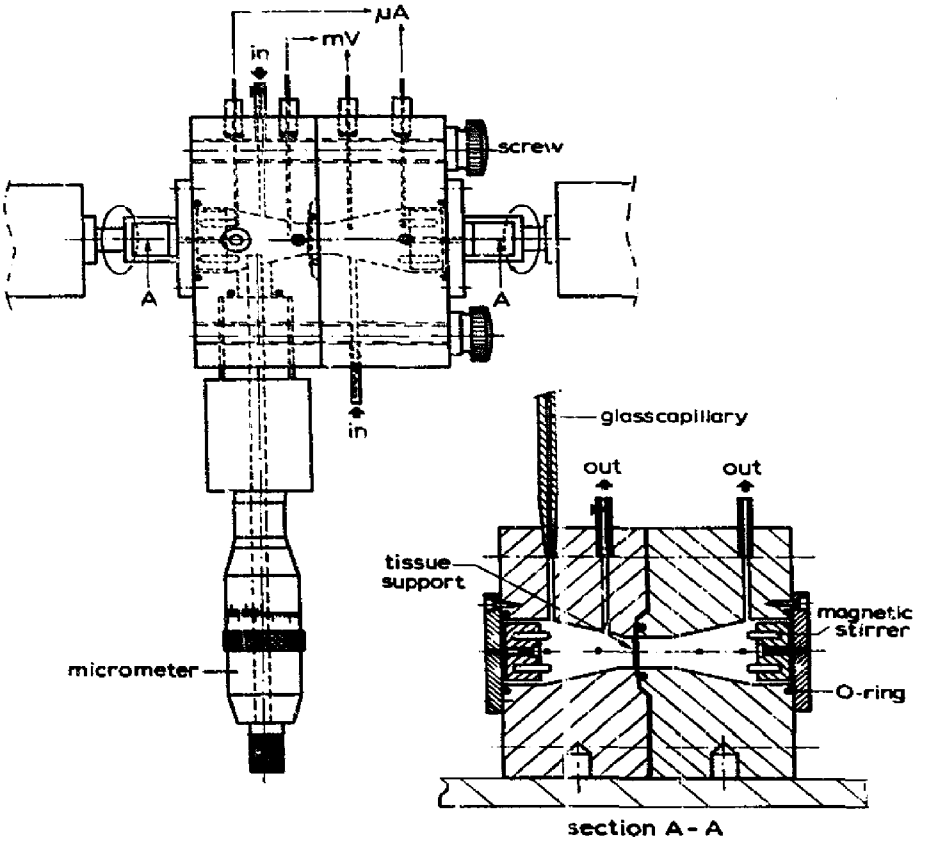

Fig. 1. Lucite chamber used for volume flow measurements across gall-bladder epithelium. The chamber is shown in horizontal and vertical cross-section.

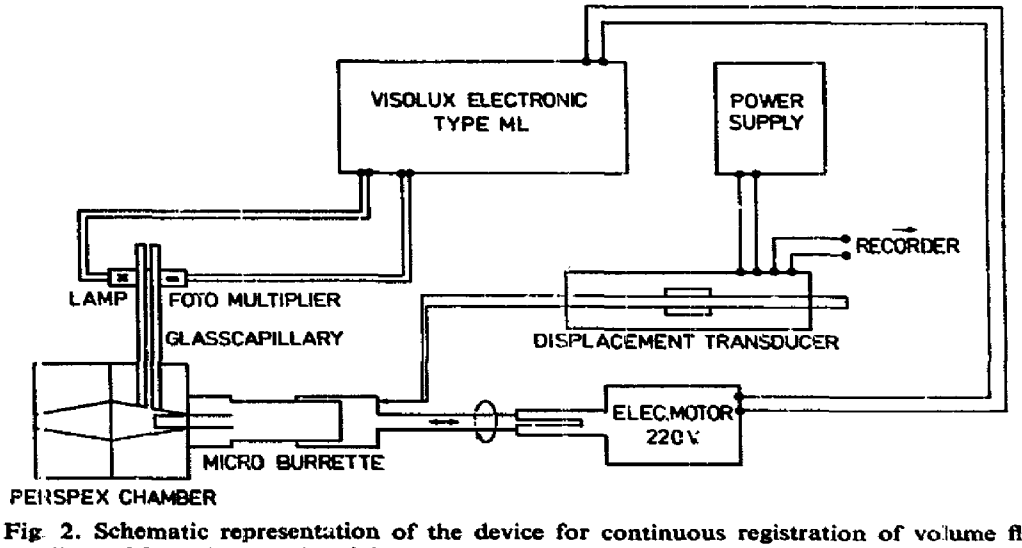

Fig. 2. Schematic representation of the device for continuous registration of volume flows. For details see Methods and Materials.

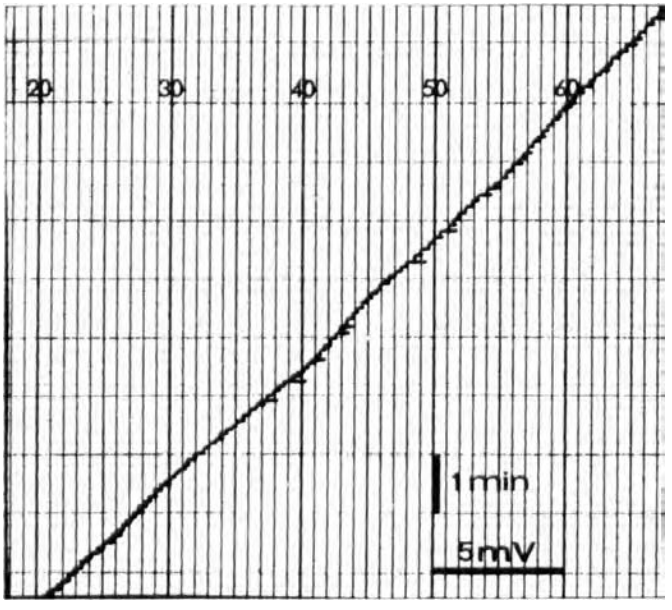

Fig. 3. Typical example of a record of volume flow registration with the device described under Methods and Materials. In this record 5 mV corresponds to 4.12 μl.

magnet (Fig. 1). In this chamber the thickness of the unstirred layers on the mucosal and serosal side was 139±11 (8 observations) and 593±32 (8 observations) μm respectively. (These values have been calculated from the half-times for the build-up of diffusion potentials as described by Smulders and Wright [7]).

Electrical measurements

The electrical potential difference across the gall bladder was measured between two calomel half-cells connected to the mucosal and serosal solution via Ringer/agar bridges. The half-cells were fed into a Keithley electrometer (Model 610 C) whose output was connected to the second channel of the pen recorder. Current could be passed through the tissue via Ag/AgCl wires. The current was drawn from a 24 V battery by means of a potentiometer. Gall bladders were selected on the magnitude of the diffusion potential when the Na concentration in the mucosal solution was reduced to half the normal value (Na replaced by choline). Gall bladders with a 2 : 1 sodium dilution potential of less than 8 mV were discarded (permeability coefficient ratio, $P_{Cl}/P_{Na} > 0.35$) [8].

Throughout this paper errors are expressed as the standard error of the mean with the number of observations in parentheses.

RESULTS

Spontaneous fluid transport rates

The rates of active fluid transport* across rabbit gall bladders, measured both

* Throughout the paper "active fluid transport" means the volume flow coupled to active transport of salt.

TABLE I

ACTIVE FLUID TRANSPORT IN RABBIT GALL BLADDER EPITHELIUM

Comparison of active fluid transport rates in rabbit gall bladders measured in chamber and in sac preparations ($t = 0$ is the time when the volume flow recording is started). Number of observations in parentheses.

	J_V in $\mu l \cdot h^{-1} \cdot cm^{-2}$	
	$t = 0$	$t = 60$ min
Chamber preparation	181.0±14.3 (15)	92.2±16.3 (7)
Sac preparation [6]	89.3± 5.5 (10)	71.4± 4.9 (10)

gravimetrically and volumetrically, are listed in Table I. The results obtained with the two methods were different, i.e. the initial flow rates were higher in chamber preparations than in sac preparations. Better stirring and oxygenation in the chamber preparation may have been responsible for this observation. Higher flow rates have also been observed across frog gall bladders mounted in chambers as compared to sac preparations (Wright, E. M., personal communication). Hence the flow rates observed in chamber preparations provide a better estimate of the transport capacity of the gall bladder. Another difference between the techniques is that active flows decay much faster in chamber preparations than in sac preparations. Addition of glucose to the bathing solutions did not abolish the rapid decay of active transport, which suggests that the decay is not due to exhaustion of endogenous substrates. Smulders et al. [2] have reported that volume flows from mucosa to serosa across gall bladders mounted in chambers induced a large increase in the volume of the submucosal spaces within the tissue. This implied an increase in hydrostatic pressure in the submucosa. It is well known that serosal positive pressures impede active volume flows in intestinal epithelium [9] and in kidney tubules [10]. Therefore the decay in active flows in chamber preparations might be due to a build-up of pressure underneath the epithelial layer. In sac preparations this phenomenon is known to be less pronounced [11]. Despite the rapid decay in active transport the chamber technique is useful in studying factors which influence active transport. Table II shows the effect of thiocyanate ions on active volume flow. In four gall bladders mounted in

TABLE II

INHIBITION OF ACTIVE FLUID TRANSPORT WITH SCN^- IONS

25 mM SCN^- was added after 10 min to both bathing solutions; the volume flow observed 25 min after addition of SCN^- is tabulated. 20–25 min after SCN^- has been washed out fluid transport rates are back to control levels (Table I). Number of observations in parentheses.

J_V in $\mu l \cdot h^{-1} \cdot cm^{-2}$		
Ringer		Ringer+25 mM SCN^-
$t = 0$	$t = 60$ min	$t = 35$ min
179.0±23.7	94.7±9.2	56.0±7.9 (4)

the chamber, addition of 25 mM SCN^- inhibits 69 % of the fluid transport. The effect was reversible within 20 min of washing away the thiocyanate. This result is comparable to observations of Sullivan and Berndt [12] with sac preparations of gall bladders.

Current-induced volume flows across non-transporting gall bladders

In this study we have used currents (I) up to 1.15 mA · cm^{-2}. These currents did not alter the magnitude of dilution potentials, implying no damage to the selectivity properties of the gall bladder. Similar currents have been used by Bindslev et al. [4], also without damage to frog gall-bladder selectivities.

Applying electrical gradients across the gall bladder led to volume flows (J_V) towards the negative electrode (Fig. 4). Passage of current across ouabain-inhibited gall bladders (10^{-3} M ouabain in the serosal solution) at 38 °C produced flow rates of the same magnitude as gradients across non-transporting bladders at 18 °C. This implies no significant contribution of temperature gradients across the tissue to volume flows, hence absence of thermo-osmosis. Between 300 and 900 μA, volume flows were linearly related to current densities in both directions. However, the slope of J_V vs. I was 3 times greater for currents to the serosa than for currents in the opposite direction (Fig. 4). This rectification phenomenon closely resembles the difference between osmotic flows to the serosa and those to the mucosa as reported by Smulders et al. [2]. Concomitantly, current from serosa to mucosa ($I > 300$ μA) caused the resistance to increase to a new value 4.6 times greater than the control value of 36.4±6.4 Ω cm^2 ($n = 10$ at 18 °C). Similar current-induced resistance changes have been reported by Bindslev et al. [4] in frog gall bladders.

When current was switched on, the steady-state flow was reached within 3-5 min. Switching off a current of 900 μA unmasked so-called polarization potentials of

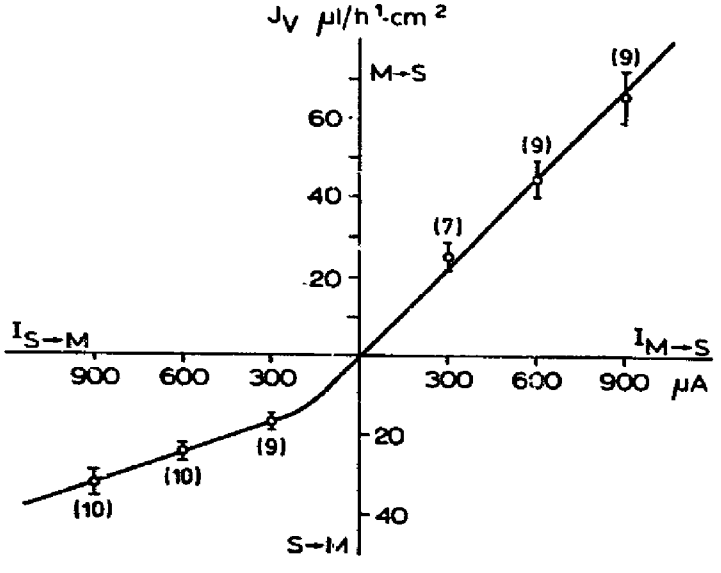

Fig. 4. Relation between imposed electrical gradients and resulting volume flows across non-transporting gall bladders. The line is drawn by eye.

at least 12 mV. These potentials decayed back to zero within 5 min. Similar polarization potentials have been reported by Wedner and Diamond [5] and the authors demonstrated current-induced volume flows across sac preparations of gall-bladder epithelium.

Current-induced volume flows across actively transporting gall bladders

Since the rate of fluid transport across gall bladders decreased rapidly during the first hour, we switched on current about 60 min after the bladder had been

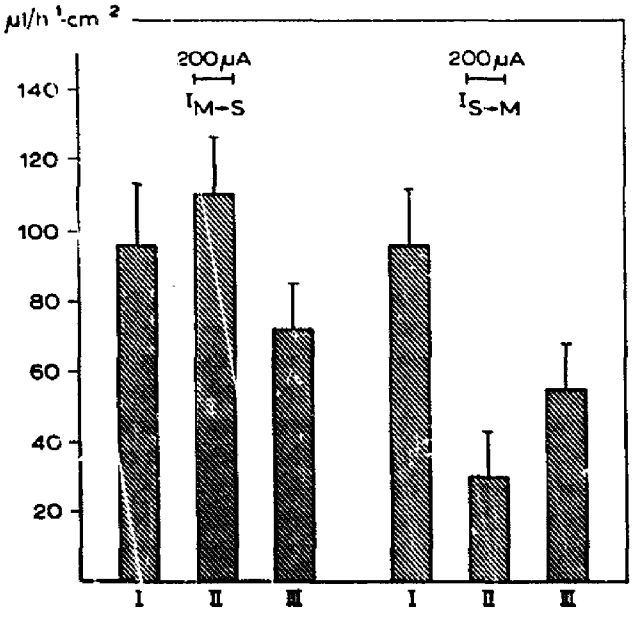

Fig. 5. Effects of 15 min current pulses of 200 μA on fluid transport rates across gall-bladder epithelium (bar II). Mean values of volume flows before and after current pulses are shown as control values (I and III). $I_{M\to S}$, current from mucosa to serosa. $I_{S\to M}$, current from serosa to mucosa.

TABLE III

COMPARISON OF VOLUME FLOWS INDUCED BY 200 μA IN ACTIVELY TRANSPORTING AND IN NON-TRANSPORTING GALL BLADDERS

Increase and decrease in fluid reabsorption rates induced by a 200 μA current from mucosa to serosa ($I_{M\to S}$) and from serosa to mucosa ($I_{S\to M}$) respectively. The average transport rate observed just before and after the current pulse of 15 min has been used as a reference. Passage of the 200 μA current through transporting gall bladders had no influence on the tissue resistance. Therefore volume flows induced across non-transporting bladders were assumed to be equal in both directions, and have been taken from Fig. 4.

	ΔJ_V in $\mu l \cdot h^{-1} \cdot cm^{-2}$	
	$I_{M\to S}$ ($P < 0.01$)	$I_{S\to M}$ ($P < 0.01$)
200 μA Transporting	28.9±3.5 (12)	−33±4.4 (8)
200 μA Non-transporting	15.0±1.7 (9)	−15±1.7 (9)

mounted in the chamber. Fig. 5 shows the effects of electrical gradients on actively transporting gall bladders. In Table III the increase and decrease in the transport rate due to a current of 200 μA is compared with flow rates induced by equal currents in non-transporting bladders. The table indicates that electrical gradients have a greater effect on actively transporting than on non-transporting gall bladders. Therefore current is able to enhance or to inhibit the active transport mechanism of gall-bladder epithelium. This result is comparable to the effect of current on volume flow across proximal tubules of *Necturus* kidney reported by Spring and Paganelli [13].

DISCUSSION

In this study we report a method for measuring volume flow across epithelial tissue and application of this method in a study of the effects of electrical gradients on volume flow across gall-bladder epithelium. The sensitivity of the method is such that it should be used when the volume flow J_V is greater than 5 $\mu l \cdot h^{-1} \cdot cm^{-2}$. The flows reported in this study were much greater than the minimum resolvable flow for which the method has been proven to be reliable.

The striking difference between active transport rates reported here and previous ones suggests that in sac preparations transport rates are smaller due to a difference in aeration. Martin and Diamond [14] reached this conclusion by observing that everted sacs consumed more oxygen than non-everted preparations. Bindslev et al. [4] suggested that when the mucosal solution is unstirred, the rate of active sodium transport might be limited by diffusion of sodium through the unstirred layer adjacent to the mucosal membrane. In frog gall bladder this would explain the absence of a potential related to transport when the mucosal solution was unstirred. Both anoxia and an unstirred layer effect may have been responsible for lower transport rates in sac preparations. In a previous study we have estimated the ratio of Na^+ ions transported per mol ATP split by the $(Na^+ + K^+)$-stimulated ATPase present in this tissue (Na^+/ATP ratio) [6]. The number of Na^+ ions transported was calculated from the rate of active fluid transport assuming isotonicity. Using the higher transport rates of this study the Na^+/ATP ratio rises by a factor of 2, from 1.0 to 2.0. The latter value is in excellent agreement with the values reported for a large variety of tissues [15], supporting the evidence that $(Na^+ + K^+)$-ATPase has a primary role in fluid reabsorption.

A disadvantage of higher flow rates seemed to be that fluid transport becomes less stable as indicated by its rapid decay. This is most probably due to a rapid build-up of hydrostatic pressure developing in the submucosal space. It has been proven that the serosal muscle and collagen layer constitute a significant barrier to bulk flow [3], hence, pressure is needed to force fluid that has been transported by the epithelium through this layer. A hydrostatic pressure will increase the back flux of water and salt into the mucosa. Serosal positive pressure has been shown to be extremely powerful in counteracting active flow. In small intestine a pressure of 5-10 cm H_2O turns absorption into secretion [9].

The inhibition of fluid transport by SCN^- ions suggests a link with an anion-sensitive ATPase activity which has been reported in some epithelia [16]. This enzyme is also inhibited by SCN^-. However, recently we could demonstrate that in rat small intestine an SCN^--inhibitable ATPase is present only in the mitochondrial

fraction and not in purified plasma membranes (van Os, C. H., Mircheff, A. M. and Wright, E. M., in preparation). Therefore it is possible that SCN^- inhibition of transport is due to interference at the level of energy production rather than at the cell membrane level.

Wedner and Diamond [5] first showed that passage of current across gallbladder sacs causes fluid to flow towards the negative electrode. In this study their original observation is confirmed and extended. Current-induced water flow is either due to electro-osmosis or to the so-called transport number effect [17, 18], i.e. osmosis due to salt polarization in unstirred layers. From the magnitude of polarization potentials observed after switching off the current, one is able to calculate how much of the current-induced flow is due to polarization effects. In our experiments a current of 900 μA, mucosa to serosa, caused a flow of 65 $\mu l \cdot h^{-1} \cdot cm^{-2}$. The osmotic water permeability of rabbit gall bladder in this chamber was $5.1 \pm 0.5 \cdot 10^{-3}$ cm $\cdot$ sec^{-1} (measured with 100 mM sucrose in the serosal solution). Therefore a gradient of 200 mosM NaCl is needed to cause a flow of 65 $\mu l \cdot h^{-1} \cdot cm^{-2}$. The reflection coefficient of NaCl is 0.81 [19] and the osmotic coefficient 0.93 [20]. Therefore 200 mosM is equivalent to a concentration difference of 132 mM NaCl. From the Goldman equation,

$$E_M - E_S = -\frac{RT}{zF} \ln \frac{P_K \gamma_K [K_M] + P_{Na} \gamma_{Na} [Na_M] + P_{Cl} \gamma_{Cl} [Cl]_S}{P_K \gamma_K [K]_S + P_{Na} \gamma_{Na} [Na]_S + P_{Cl} \gamma_{Cl} [Cl]_M},$$

(permeability coefficients $P_K : P_{Na} : P_{Cl} = 1.9 : 1 : 0.2$ [21]; γ_K, γ_{Na} and γ_{Cl}, activity coefficients: 0.74) [20], we calculate a potential difference of 10.0 mV for a concentration difference of 132 mM NaCl. Since the unstirred layer on the serosal side is five times thicker than on the mucosal side, most of the polarization takes place in the serosal unstirred layer. This leads to a junction potential of 3.2 mV maximally, with the same polarity as the polarization potential. Hence, if all the current-induced volume flow were due to salt polarization we should observe a polarization potential difference of about 13 mV. The polarization potentials, observed 5 s after switching off current, were on the average about 12 mV. The initial value would still be higher in view of the fast decay of polarization potentials*.

Therefore we can conclude that all of the current-induced volume flow can be ascribed to salt polarization in the unstirred layers. This conclusion is supported by the observation that flow reached a steady state 3 min after switching on current. If electro-osmosis were the cause of water flow one would expect an instantaneous flow without transients [17, 18]. From their experiments, Wedner and Diamond [5] concluded that 80 % of the flow was due to polarization. In plant cells (*Chara australis*) about 60 % of current-induced flows could be attributed to the transport number effect [18]. An important implication of our results on current-induced flows is that streaming potentials in the gall bladder observed during osmotic flows, are solely due to concentration polarization in the unstirred layers. In this study we have

* During passage of current from serosa to mucosa the potential drop between the electrodes is on average 350 mV (the tissue resistance under this condition is about 150 $\Omega \cdot cm^2$, hence the actual potential across the tissue is 180 mV. The rest of the potential drop is across the fluid resistance between the electrode tips). After switching off current it took us 5 s to readjust the voltmeter and to measure the polarization potential. Therefore the value of 12 mV is an underestimated value.

shown that flows were linearly related to current densities. This is in good agreement with the observation that the magnitude of polarization potentials was also linearly related to current densities [4]. In addition, rectification of water flow completely parallels rectification of current. Both phenomena are probably due to a collapse of the lateral intercellular spaces as described by Smulders et al. [2] and Bindslev et al. [4].

Besides current-induced flows due to polarization, we found an effect of current on active fluid reabsorption. The effects we observed were qualitatively similar to those reported by Spring and Paganelli [13] in *Necturus* proximal tubules. These authors were able to demonstrate a linear relationship between the transtubular potential difference and the net Na^+ flux. Comparable experiments have been done with tight epithelia. In frog skin as well as in toad bladder, the rates of net sodium transport and oxygen consumption increased with decreasing transmural potential difference values [22, 23]. It should be noted, however, that the mechanisms by which current affects net sodium transport in tight and leaky epithelia must be totally different. In tight epithelia passage of current greatly influences the potential profile of the epithelium and it therefore interferes directly with the energetics of sodium entry and extrusion. In gall bladder 96 % of the current bypasses the cells [24]. With the currents we used, the potential difference values across both cell membranes are changed by 4 mV at most. This is negligible compared to the membrane potentials of 60–70 mV in ref. 25. It is more likely that passage of current across gall bladder interferes directly with the standing gradients in the lateral intercellular spaces.

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REFERENCES

1 Diamond, J. M. (1962) J. Physiol. 161, 442–473
2 Smulders, A. P., Tormey, J. McD. and Wright, E. M. (1972) J. Membrane Biol. 7, 164–197
3 Wright, E. M., Smulders, A. P. and Tormey, J. McD. (1972) J. Membrane Biol. 7, 198–219
4 Bindslev, N., Tormey, J. McD., Wright, E. M. (1974) J. Membrane Biol. 19, 357–380
5 Wedner, H. J. and Diamond, J. M. (1969) J. Membrane Biol. 1, 92–108
6 van Os, C. H. and Slegers, J. F. G. (1971) Biochim. Biophys. Acta 241, 89–96
7 Smulders, A. P. and Wright, E. M. (1971) J. Membrane Biol. 5, 297–318
8 Barry, P. H. and Diamond, J. M. (1970) J. Membrane Biol. 3, 93–122
9 Hakim, A. A. and Lifson, N. (1969) Am. J. Physiol. 216, 276–284
10 Early, L. E. and Schrier, R. W. (1973) in Handbook of Physiology Section 8: Renal Physiology (Orloff, J. and Berliner, R. W., eds.), pp. 721–762, American Physiological Society, Washington
11 Tormey, J. McD. and Diamond, J. M. (1967) J. Gen. Physiol. 50, 2031–2060
12 Sullivan, B. and Berndt, W. O. (1973) Am. J. Physiol. 225, 838–844
13 Spring, K. R. and Paganelli, C. V. (1972) J. Gen. Physiol. 60, 181–201
14 Martin, D. W. and Diamond, J. M. (1966) J. Gen. Physiol. 50, 295–315
15 Bonting, S. J. (1970) in Membrane Metabolism and Ion Transport (Bittar, E. E., ed.), Vol. I, pp. 286–364, Wiley-Interscience, London
16 Kasbekar, D. K. and Durbin, R. P. (1965) Biochim. Biophys. Acta 105, 472–482
17 Barry, P. H. and Hope, A. B. (1969) Biophys. J. 9, 700–728

18 Barry, P. H. and Hope (1969) Biophys. J. 9, 729–757
19 van Os, C. H., de Jong, M. D. and Slegers, J. E. G. (1974) J. Membrane Biol. 15, 363–382
20 Robinson, R. A. and Stokes, R. H. (1970) Electrolyte Solutions, 2nd edn., Butterworths, London
21 Wiedner, G. and Wright, E. M. (1975) Pflügers. Arch 358, 27–40
22 Vieira, F. L., Caplan, S. R. and Essig, A. (1972) J. Gen. Physiol. 59, 77–91
23 Mandel, L. J. and Curran, P. F. (1973) J. Gen. Physiol. 62, 1–24
24 Frömter, E. (1972) J. Membrane Biol. 8, 259–301
25 van Os, C.H. and Slegers, J. F. G. (1975) J. Membrane Biol. 24, 341–363