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Electrical Resistance of Rabbit Submaxillary Main Duct: A Tight Epithelium with Leaky Cell Membranes

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Summary. The electrical resistance of rabbit salivary main duct epithelium has been measured. A small axial electrode, which passed current and measured potential simultaneously, was placed inside the ductal lumen. A cylindrical spiral was wound around the main duct and served as outside current electrode. The instantaneous current voltage relations were linearly up to current densities of 1.5 mA/cm^2, independently of the Cl concentration in the bathing solutions. Strong polarization effects were observed in low Cl solutions. There was a significant inverse correlation between the spontaneous potential difference across the epithelium and the epithelial resistance in solutions with either high or low Cl concentrations. In high Cl solutions the epithelial resistance was 12.2 ± 1.8 ($n=7$) Ωcm^2. The resistance increased when the mucosal Na and Cl concentrations decreased. After addition of ouabain the resistance always decreased. The temperature dependence of the resistance was determined, and apparent activation energies were calculated. Values for activation energies ranged from 3.2 to 6.5 kcal/mol, depending on the ionic composition of the bathing solutions. Addition of amiloride to the mucosal solution led to an increase in resistance by a factor of 2.1 in high Cl solutions and of 4.1 in low Cl solutions. When ouabain was applied before amiloride, there was no effect on the resistance in high Cl solutions and a smaller increase in the resistance in low Cl solutions. The results of this study support the conclusion that the low resistance of main duct epithelium resides in the cell membranes and is not due to a paracellular pathway.

Salivary glands are composed of two functionally different epithelial structures, i.e., a secretory part secreting salt and water isotonically and a ductal part reabsorbing salt hypertonically. The main duct of rabbit submaxillary gland attracts attention because of its unusual combination of electrical properties. The potential difference across ductal epithelium can be as high as 150 mV in solutions with SO_4 as major anion, while in Cl-containing solutions the epithelial resistance is as low as the re-

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sistance of proximal tubules of the kidney (Knauf, 1972*a*, *b*; Frömter *et al.*, 1974). Despite the low resistance the transport of NaCl is hypertonic (Martin *et al.*, 1972). Due to these paradoxal properties, it is hard to classify ductal epithelium of rabbit salivary glands either as a tight or a leaky epithelium (Augustus *et al.*, 1977).

Determination of epithelial resistance in salivary ducts, by applying cable theory, has presented two problems. On sending current through the tissue, strong polarization effects were observed, and in Cl-solutions application of cable theory became doubtful since the length constant λ approached the dimension of the duct diameter (Knauf, 1972*a*; Frömter *et al.*, 1974).

Another approach was to calculate the resistance from unidirectional Cl fluxes, since the main part of the conductance is a Cl-conductance, but much higher resistance values were obtained than by application of cable theory (Slegers *et al.*, 1975). We decided to use a direct method to measure the resistance of main duct epithelium. Therefore a small axial electrode was constructed and placed inside the lumen of the duct. The electrode could pass current and measure the voltage in the lumen simultaneously.

This study confirmed that in Cl solutions the resistance of rabbit maxillary main duct is as low as $10\,\Omega cm^2$, in agreement with results obtained from cable analysis. We also found evidence that the low resistance is located in the cell membranes, and the extracellular route must have a relatively high resistance. Therefore, rabbit main duct epithelium can be classified as a "tight" epithelium with "leaky" cell membranes.

Materials and Methods

Main ducts of submaxillary glands of white rabbits were dissected and mounted on two perfusion pipettes in a perfusion chamber as described before (Knauf, 1972*a*, Augustus, 1976). The bottom of the perfusion chamber was made of a silver plate, which assured good thermal contact between the bathing fluid, in which the duct was suspended, and a Peltier element (Sirigor, Siemens). The temperature of the bathing fluid was controlled by regulating the Peltier element voltage (for details *see* Augustus & Cuperus, 1977).

Resistance Measurement

A combined current-passing and voltage-sensing axial electrode was placed inside the lumen of the duct through one of the perfusion pipettes. The axial electrode consisted of a glass capillary (OD $\sim$ 100 μm) with a closed tip. The outside surface of the capillary

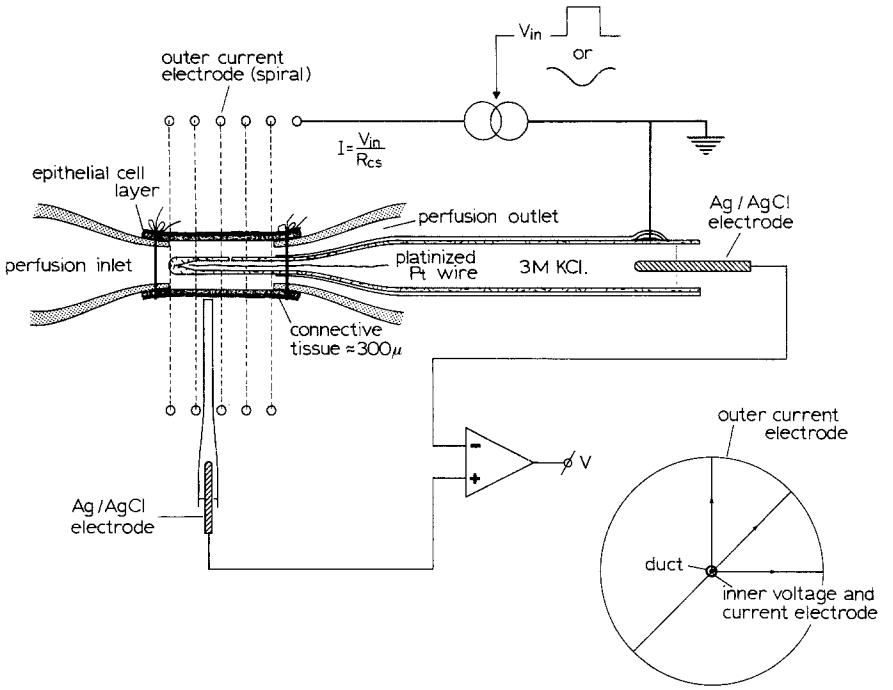

Fig. 1. Schematic drawing of the experimental set-up for measuring the transepithelial resistance and potential difference. In the right corner a cross section orthogonal to the duct axis is given. V_{in} is the input voltage to the voltage controlled current source. The current of this source is given by $\frac{V_{in}}{R_{cs}}$ where R_{cs} is a fixed resistor of the current source

was coated with an Ag/AgCl layer which served as a current electrode. A hole of 20-μm diameter etched in the glass wall 3 mm from the closed tip assured electrical contact between the 3-M KCl solution inside the capillary and the perfusion fluid inside the lumen of the duct. Salt leakage out of the KCl-filled capillary electrode through the 20-μm hole could possibly influence the epithelial parameters. However, the lumen was continuously perfused (12 μl/min), and we could not detect any concentration change in the effluent perfusion solution. Details of construction of this electrode and its electrical properties have been published (Augustus & Bijman, 1977). An Ag/AgCl coated cylindrical spiral was wound around the main duct (7 turns, OD 1 cm) and served as the serosal current electrode. Another Ag/AgCl electrode was connected to the serosal bathing fluid via a 3-M KCl salt bridge and was used for reference potential. The arrangement of current and voltage electrodes is shown in Fig. 1. The experimental procedure for measuring the resistance and the potential difference across the main duct is shown in Fig. 2.

Bipolar current pulses, consisting of a positive and negative pulse of 30 msec (Grass S-48) combined by two Grass stimulus isolation units (I-45), were used to measure the epithelial resistance. This bipolar voltage pulse forms the input of a voltage-controlled current source (Analog 44 K). The rise time of the current pulse was 0.2 μsec. The transepithelial potential difference (ψ_{SM}) and the voltage deflections due to current pulses were measured with a differential amplifier (Analog 603 K).

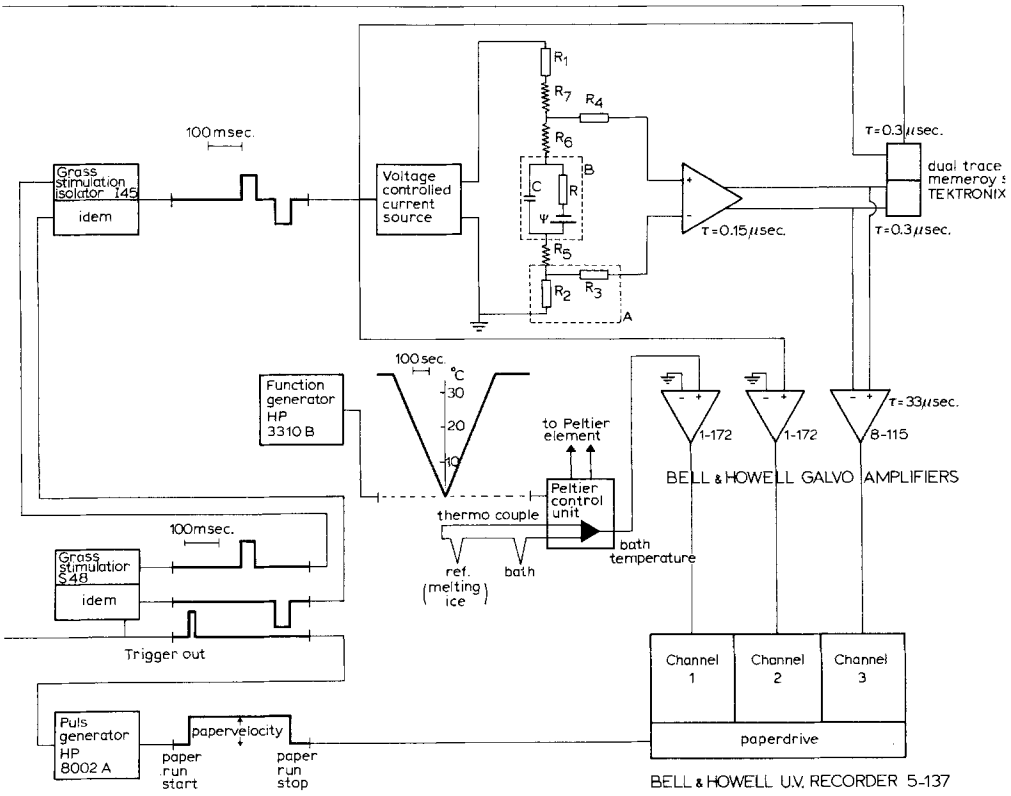

Fig. 2. A block diagram of the electrical equipment used for measuring the resistance and the potential. In *A* an equivalent scheme of the combined current voltage electrode: R_2 is the impedance of the outer current part and R_3 the impedance of the inner voltage part. R_5 is the Ringer's series resistance between the axial electrode and the luminal membrane. In *B* an equivalent scheme for the epithelial cell layer. R_6 is the resistance of the connective tissue between the serosal membrane and R_4 the serosal voltage electrode. R_7 is the Ringer's resistance between the connective tissue and outer current electrode which has impedance R_1. For further details *see* text

The influence of temperature on ductal resistance was studied as follows. The temperature of the bathing solution was varied linearly from 35 to 0 °C and vice versa by means of changing the input voltage of the control unit of the peltier element. Input voltage of the control unit was generated by a HP function generator (3310 B). Bath temperature, the bipolar current pulse, and ψ_{SM} were recorded on a UV galvanometer recorder (Bell and Howell type 5–137), 3 dB point of the recording system was 5 kHz. The bipolar pulse and the voltage deflections were also monitored on a dual beam storage oscilloscope (Tektronix 3A3). Before starting the bipolar current pulse, the paper velocity of the recorder was increased to 30 cm/sec by a stimulus from a pulse generator (Hewlett Packard 8002A), which in turn was triggered by a pretrigger pulse of the Grass stimulator (*see* Fig. 2).

The resistance of the epithelium R was calculated from the voltage deflection, ΔV, caused by the current ΔI. In order to compare resistance values with values reported in the literature, R was multiplied with the surface area of the epithelium to get the specific

resistance R_{SM} in Ωcm^2. During a perfusion velocity of 12.5 µl/min, used in this study, the main duct diameter was 297 µm (Slegers *et al.*, 1975). Since the duct is mounted on perfusion pipettes with strings, we can define two lengths: h, the length between the tips of the perfusion pipettes, and $h+2l$, where l is the length between the string and the open end of the pipette (Fig. 11 in the *Appendix*).

In the *Appendix* we analyzed the length problem in detail and derived an implicit function for the resistance of ductal epithelium, which takes into account the edge effects on the current clamp, the resistance of solutions between the voltage electrodes, and the resistance of the connective tissue layer.

Perfusion and Bathing Fluids

The solution used to perfuse the main duct had the following composition (in mM): Na, 144; K, 4; Mg, 1; Cl, 100; HCO_3^-, 25; acetate, 10; pyruvate, 10; SO_4, 2.5. After gassing with 95% O_2 and 5% CO_2, the pH was 7.4. Sulphate ions were used to replace Cl ions, and mannitol was added to maintain isotonicity. Na ions were replaced by K ions. The serosal bathing solution was similar to the perfusate but contained in addition 6 mM glucose and 3% Haemaccel (plasma expander). The solutions contained minimally 2 mM Cl, and in this Cl concentration all Ag/AgCl electrodes were functioning properly. Throughout this paper we refer to low and high Cl solutions, which stand for solutions containing either 2 or 100 mM Cl, respectively.

Results

Current-Voltage Relations and Polarization Effects

In preliminary experiments on rabbit main ducts we observed three voltage transients upon passing current through the epithelium when the tissue was bathed in low Cl solution. The first one had a time constant much smaller than one msec. The other two transients had RC values of 0.2 and 2 sec. The fast transient represented the capacitance of the epithelium, while the slower ones are related with polarization effects. To measure the resistance we used pulses of 30 msec duration, and in this time span the polarization transients were considered as a linear function of time. Therefore, the instantaneous voltage response ΔV due to a current pulse ΔI was found by extrapolating ΔV to $t \to 0$. Fig. 3 shows voltage responses to current pulses. The polarization effects decrease with increasing Cl concentrations. In low Cl solutions the polarization is more pronounced when current is flowing from serosa to mucosa ($I_{S \to M}$) than during current flow in the opposite direction (Fig. 3, 2-2 Cl). However, upon cooling the tissue to 0 °C this asymmetry disappears, which is shown in Fig. 4*b*, where $\Delta \psi_{SM}$ at the end of 30 msec of current flow is plotted against ΔI_{SM}. Thus at 35 °C the polarization is asym-

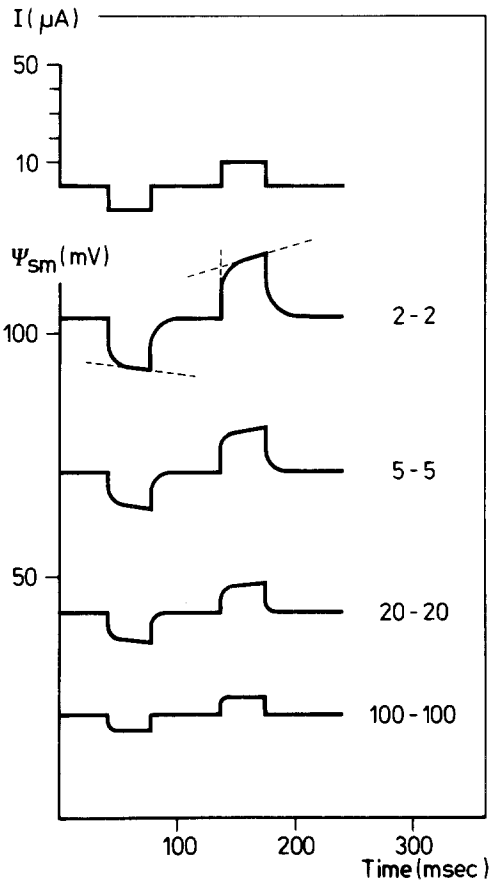

Fig. 3. Voltage deflections superposed on the open-circuit transepithelial PD as response to a bipolar current pulse in different symmetrical chloride Ringer's of which the chloride concentration (in mmol) is given in the right of the figure. Results are redrawings of the original registrations. The point between outersection of the dotted lines to the base line in the R_2 case is equal to IR, the steady-state value of the electrical transient. For further details *see* text

metrical while at 0 °C the asymmetry disappeared, but at the same time the nonlinearity increased. This observation strongly suggests that the active transport mechanism interfered with the polarization induced by the current. The polarization effects are most likely due to transport number effects causing concentration polarization in unstirred layers adjacent to the plasmamembranes of the epithelium. The polarization is the most pronounced in SO_4 solutions, a situation in which the epithelium behaves asymmetrically towards changes in ion concentrations of the bathing solutions (Knauf, 1972*a*). For a detailed description of the polarization effects other experiments are needed. In this study we

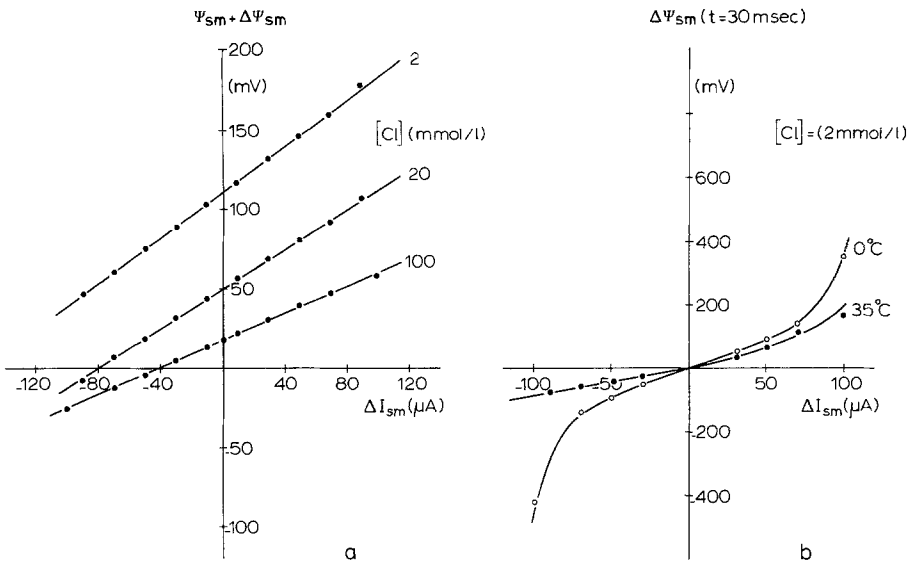

Fig. 4. (*a*): Instantaneous current voltage relations in symmetrical solutions with varying Cl concentrations. (*b*): Current voltage relations at 0 and 35 °C where the voltage deflection was sampled at the end of a 30 msec current pulse

concentrate on the resistance of ductal epithelium. By extrapolation of $\Delta\psi_{SM}$ to $t \rightarrow 0$ we observe that all current-voltage relations are linear, hence independent of the composition of the bathing solutions. These linear $I-V$ relations prove that the extrapolation procedure has removed secondary polarization effects.

Relations between Epithelial Resistance and Open-Circuit Potentials

In low Cl solutions the spontaneous transepithelial PD varied between 100 and 145 mV, while the epithelial resistance ranged from 7.5 to 60 Ωcm^2. In Fig. 5 values for ψ_{SM} measured immediately after mounting of the duct in the perfusion chamber are plotted against the resistances of the same ducts. There is a significant inverse correlation between ψ_{SM} and R_{SM} (Kendall's rank correlation test). Increasing Cl concentrations lowered the PD as well as R_{SM}, but the inverse correlation remained. A similar inverse relation was described for extremely tight epithelia with resistance values three orders of magnitude greater than the resistance of main duct epithelium (Higgins *et al.*, 1975; Lewis, Eaton & Diamond, 1976). Evidently an inverse relationship between the PD and resistance does not

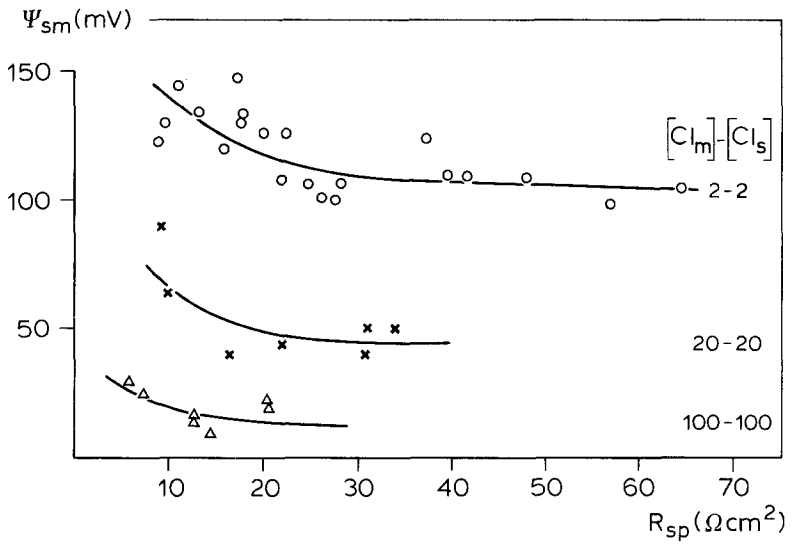

Fig. 5. Relationship between spontaneous transepithelial PD, ψ_{SM}, and epithelial resistance, R_{SM}. According to Kendall's rank correlation test, there is a significant inverse correlation between ψ_{SM} and R_{SM} in all three solutions

occur in leaky epithelia, where a low extracellular shunt resistance reduces the transepithelial PD dramatically (Schultz, 1972).

Ionic Dependence of the Resistance

The influence of Cl ions on R_{SM} was determined in 7 ducts starting in low Cl solutions symmetrically applied. The result is given in Fig. 6. There is a log-linear relationship between the Cl concentration and R_{SM}. However, when the concentration of Cl is raised above 50 mM, the fall in resistance becomes more pronounced. Surprisingly, addition of ouabain led to a decrease in resistance of 31% ($n=5$) in low Cl media and of 10% ($n=4$) in high Cl solutions. The first resistance measurement was done 20 sec after ouabain application. Then the resistance was sampled every 20 sec. The resistance decreased between 20 and 500 sec, and after reaching a new lower steady-state value no further decrease was observed during a period of 30 min. This observation indicates that the drop in resistance is not due to cell lysis or irreversible damage of the epithelium. Since addition of ouabain will lead to dissipation of the Na and K gradients across the cell membranes, with concomitantly an increase in cellular Cl concentration, it is likely that changes in ionic

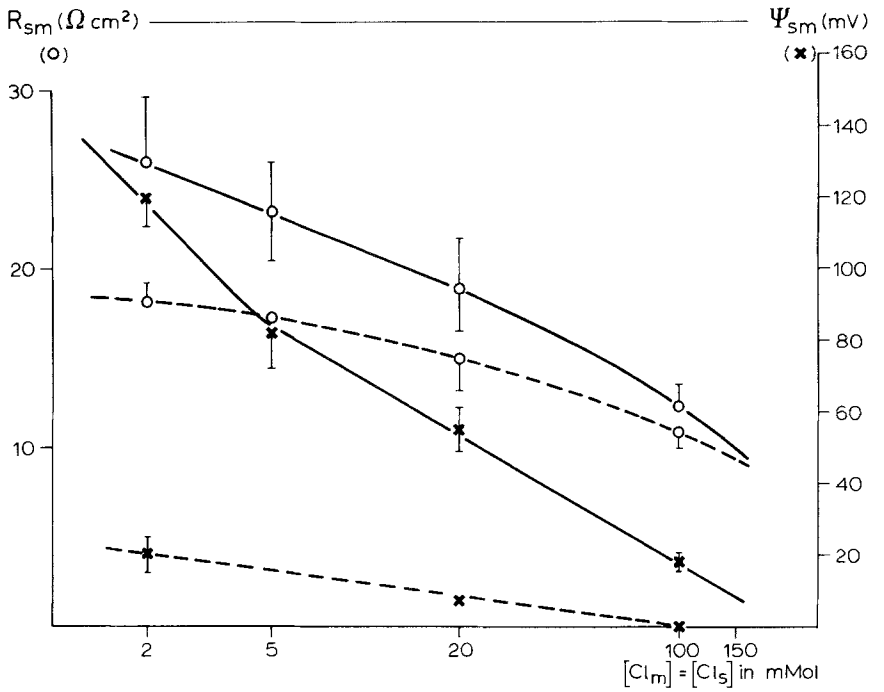

Fig. 6. The dependence of main duct resistance R_{SM} and potential, ψ_{SM}, on the Cl concentration of the bathing solutions. Solutions without ouabain (——); solutions with 10^{-4} M ouabain (– – –)

concentrations in the cellular compartment are responsible for the decrease in resistance due to ouabain. With low resistive membranes as in ductal epithelium (*see Discussion*) the change in concentrations will be a rather fast process.

The simultaneously measured PD is also linearly dependent on log [Cl], which was reported already (Frömter *et al.*, 1974; Slegers *et al.*, 1975). However, lowering the Cl concentration below 5 mM resulted in a steeper slope than what would be expected if the change in PD is only due to a Cl diffusion potential. After ouabain application, ψ_{SM} dropped immediately and reached a new steady-state within 3 to 4 min. In this situation ψ_{SM} was only slightly sensitive to Cl ions (10 mV/decade). These observations imply that the change in ψ_{SM} upon increasing the Cl concentration is rather complex and cannot simply be interpreted in terms of a diffusion potential due to the Cl selectivity of the main duct wall (*see Discussion*).

The effect of luminal Na concentration, Na_M, on R_{SM} was measured in low Cl solutions. This result is shown in Fig. 7. Also in these

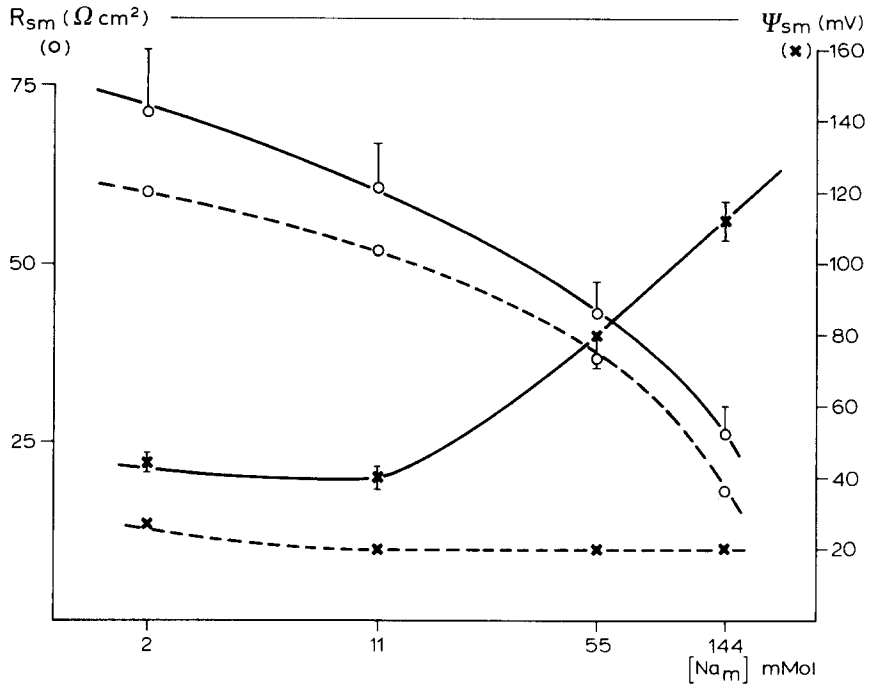

Fig. 7. Dependence of main duct resistance, R_{SM}, and potential, ψ_{SM}, on the mucosal Na concentration. Solutions without ouabain (——); solutions with ouabain (– – –)

experiments ouabain induced a drop in the resistance comparable to those in Fig. 6. The relation between Na_M and ψ_{SM} is again rather complex. Between 55 and 144 mM the slope is 80 mV/decade, while no effect on ψ_{SM} is seen between 2 and 11 mM. After ouabain application the voltage response to increasing Na concentrations is completely abolished, as was reported previously by Knauf (1972*a*).

Temperature Dependence of the Resistance

The effect of temperature on ductal resistance was measured by cooling the bathing fluid continuously from 35 to 0°C in 300 sec. After reaching zero the bath was warmed again to 35°C in the same time period. The resistance was sampled every 20 sec. In Fig. 8*A* such an experiment is shown. A so-called hysteresis phenomenon was observed. During cooling the resistance was always greater than during warming. When the tissue was treated with ouabain first, then the hysteresis disappeared (Fig. 8*B*). The ouabain curve in Fig. 8*B* proves that the

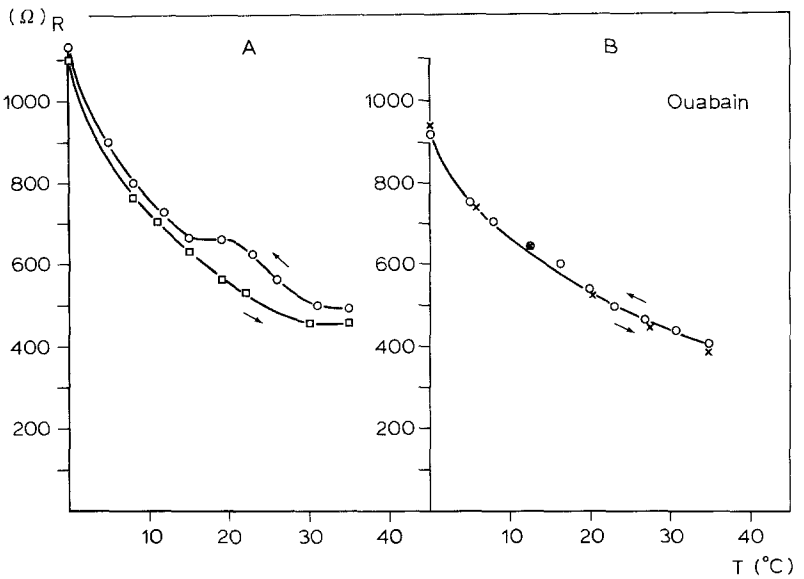

Fig. 8. Resistance of main duct epithelium as a function of bath temperature. (*A*): o – o, values measured during cooling from 35 to 0°C. □ – □, values measured during warming from 0 to 35°C. This experiment was done in low Cl solutions, but it represents a typical experiment. The hysteresis was observed in all experiments, independently of the composition of the bathing solution. (*B*): The experiment in *A* was repeated after addition of ouabain to the serosal bathing solution. o – o, values measured during cooling; × – ×, values measured during warming

hysteresis is not due to a time delay in temperature equilibration of the membranes in the tissue. It suggests that the hysteresis is related to changes in intracellular concentrations. During cooling of the main duct to 0°C, the intracellular concentrations change in a way similar to after adding ouabain. Since addition of ouabain led to a decrease in resistance, it is understandable that the resistance was lower during warming than during cooling. If the hysteresis was due to a time delay in temperature equilibration, it should be the other way around; a lower resistance during cooling and a greater resistance during warming. When the ionic concentrations are changing after a step in temperature, it is clear that resistance measurements under steady-state conditions include two effects, namely, a primary temperature effect and a secondary effect due to concentration changes. In Fig. 8*B* the resistance at 0°C in the presence of ouabain represents a steady-state measurement. When the resistance in Fig. 8*A* at 0°C is determined under steady-state conditions, the same value as in Fig. 8*B* is found. This means that measurements under steady-state conditions underestimate the temperature effect on resis-

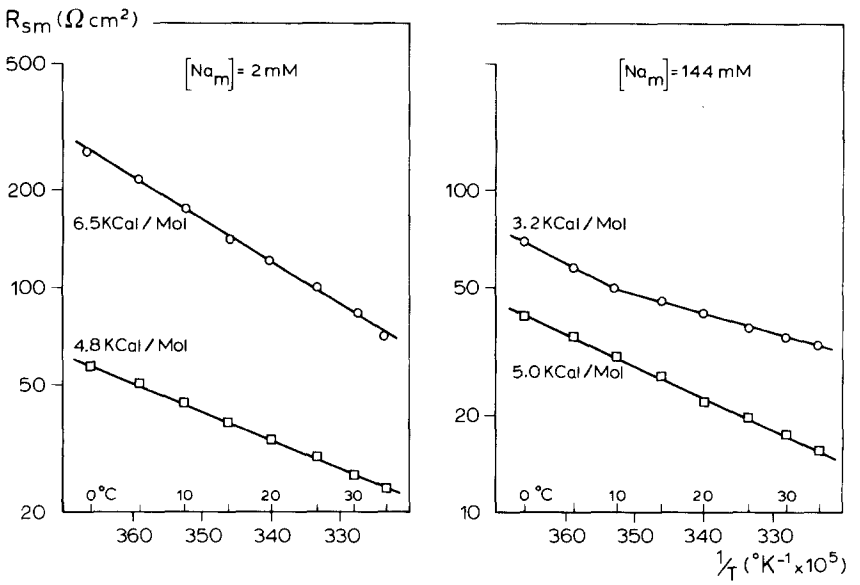

Fig. 9. Arrhenius plots of main duct resistance. Each plot represents the mean values of three tissues. The temperature dependence was determined in four different solutions:
○–○ (2 mM [Na_M]), 2–2 mM [Cl_M], [Cl_s]
□–□ (2 mM [Na_M]), 100–100 mM [Cl_M], [Cl_s]
○–○ (144 mM [Na_M]), 2–2 mM [Cl_M], [Cl_s]
□–□ (144 mM [Na_M]), 100–100 mM [Cl_M], [Cl_s]

tance by a factor of 20%. In order to minimize the secondary effect of temperature changes we have used the mean values measured during cooling and warming at the same temperature for construction of Arrhenius plots. In Fig. 9 four Arrhenius plots obtained in four different solutions are given. The apparent activation energy of the resistance in high Cl solutions is independent of the mucosal Na concentration. In the equivalent circuit of the epithelium (*see Discussion*, Fig. 10*b*) the Cl pathway is a parallel element, hence in high Cl solutions the Cl pathway is the lowest resistive element. Therefore, the apparent activation energy of the Cl pathway is about 5.0 kcal/mol. In low Cl solutions with 144 mM Na in the perfusate, the apparent activation energy is 3.2 kcal/mol. With low Na concentrations this value becomes 6.5 kcal/mol. In low Cl solutions the equivalent circuit of the epithelium reduces to a Na and K conductor in series (Fig. 10*b*). It is likely that in low Na the Na conductor dominates, hence its apparent activation energy is about 6.5 kcal/mol. With Na ions present the K conductor might be the dominating element with an activation energy of 3.2 kcal/mol. Activation energies for resistances of biological membranes have been reported as low

as 2.1 kcal/mol in muscle fiber (Del Castillo & Machne, 1953). For the resistance of aplysia nerve cell membrane activation energies of 4.2 kcal/mol in high K solutions and of 8.3 kcal/mol in high Na solutions were reported (Marchiafova, 1970). These values are close to our values observed for main duct epithelium.

The Effect of Amiloride

The natriuretic drug amiloride is known as a reversible inhibitor of Na entry into the cells across the mucosal or outer membranes of tight epithelia such as frog skin (Nagel & Dörge, 1970; Biber, 1971), toad urinary bladder (Bentley, 1968), or rabbit colon (Frizzell, Koch & Schultz, 1976). In low Cl solutions 10^{-4} M amiloride in the luminal fluid led to an increase in R_{SM} by a factor of 4.1 (Table 1). This relative increase in resistance is identical to amiloride treatment of frog skin bathed in SO_4 solutions where the resistance increased by a factor of 3.1 (Erlij, 1976). In high Cl solutions the increase in resistance was by a factor of 2.1, again comparable to amiloride effects on frog skin and urinary bladder Lewis & Diamond, 1975; Higgins *et al.*, 1975; (Erlij, 1976). Surprisingly, when ouabain was applied before the addition of amiloride, there was no effect on R_{SM} in high Cl solutions and a smaller increase in R_{SM} in low Cl solutions (Table 1). This observation suggests that the resistance increase after amiloride treatment in high Cl solutions is not directly related to a blocking of the Na conductance in the luminal membrane. Treatment with amiloride apparently results in a decrease of the intracellular Na and Cl content, because the active Na transport mechanism in the serosal membrane will continue to pump NaCl out of the cell. When this mechanism is blocked by ouabain, addition of amiloride no longer has an effect on the cellular concentrations. Therefore, the change in resistance observed in low Cl solutions with ouabain

Table 1. Influence of amiloride on epithelial resistance of rabbit main duct

$[Cl_M]-[Cl_s]$ (mM)	R_{sm} (Ωcm^2)	R_{sm} (Ωcm^2)	(amiloride, 10^{-4} M)
2–2	22.3 ± 1.8	91.4 ± 12.6	($n=7$)
100–100	8.2 ± 1.4	14.4 ± 0.9	($n=6$)
2–2 (10^{-4} M ouabain)	24.7 ± 3.6	42.2 ± 7.2	($n=4$)
100–100 (10^{-4} M ouabain)	11.8 ± 3.9	13.9 ± 4.5	($n=4$)

Table 2. Effects of asymmetrical transepithelial Cl concentrations on the resistance of main duct epithelium

Mucosal Cl concentration		Serosal Cl concentration R_{sM} in Ωcm^2		
		2 mM	100 mM	
2 mM	NS	23.5±1.9 ($n=11$) $P<0.01$ 22.0±1.9 ($n=10$) (+ouabain) $P<0.01$	19.6±1.2 ($n=11$) 13.6±1.8 ($n=9$)	$P<0.01$
100 mM	NS	12.5±1.1 ($n=7$) $P<0.01$ 15.2±1.5 ($n=11$) (+ouabain)	9.2±0.7 ($n=7$) 11.2±1.4 ($n=10$)	NS

NS not significantly different; P values according to students t test.

present can be ascribed to amiloride blocking of the Na conductance. In high Cl solutions the Na conductance must be relatively small since an amiloride effect on this pathway is undetectable when ouabain is present.

Transepithelial Ionic Asymmetries

Sodium ions have an effect on R_{SM} and ψ_{SM} only from the luminal side. On the contrary, K ions exert their effect only from the serosal side. This asymmetrical behavior towards cations has been described previously (Knauf, 1972*a*). So far we have applied Cl ions strictly symmetrically. However, in the absence of ouabain, Cl ions in the luminal fluid have more effect on R_{SM} than Cl in the serosal bath (Table 2). This asymmetry also holds for the effect on ψ_{SM} (Frömter *et al.*, 1974; Slegers *et al.*, 1975). When ouabain is present, ductal epithelium behaves symmetrically towards Cl ions, which is shown in Table 2. This observation indicates that the epithelium is symmetrical with respect to Cl ions, but the effect of Cl from the serosal side is masked by the active transport mechanism. In ducts not inhibited by ouabain it may be possible that the electrochemical gradient for Cl is in the direction cell to serosal fluid. It is also possible that leakage of Cl into the cell is balanced by active NaCl extrusion.

Discussion

For the epithelium of rabbit submaxillary main duct we measured, by means of direct method, a resistance of 12.2±1.8 ($n=7$) Ωcm^2 when the

Table 3. Epithelial resistance of rabbit submaxillary main duct

Solutions		References
Cl-Ringer's	SO_4-Ringer's	
R_{SM} in Ωcm^2		
10.2 ± 1.0 ($n=7$)	30.3 ± 4.0 ($n=7$)	This paper. Direct method instantaneous $I-V$ relation
9.6 ± 2.7 ($n=11$)	54.0 ± 3.0 ($n=5$)	Frömter *et al.* (1974) cable analysis, 5 msec current pulses
11.2 ± 1.6	68 ± 8	Knauf (1972) cable analysis, 2 min current pulses
48	454	Slegers *et al.* (1975) unidirectional Cl fluxes ($S \rightarrow M$)

tissue was bathed in symmetrical solutions containing 100 mM Cl. The lowest value observed in this situation was $6.2\,\Omega cm^2$. From Fig. 6 we can extract a value for the resistance in 150 mM Cl by extrapolation. By doing so we find $R_{SM} \simeq 10\,\Omega cm^2$, which is in good agreement with reported values obtained from one-dimensional cable analysis (*see* Table 3). In SO_4 solutions cable analysis yielded higher resistances than our direct method (Table 3). The difference must be due to the strong polarization effects. Reducing the current pulse length from 2 min to 5 msec reduced the resistance from 68 to $54\,\Omega cm^2$. From instantaneous I–V relations we arrive at $26 \pm 3.6\,\Omega cm^2$ in low Cl solutions (Table 3). Since we needed 2 mM Cl for the Ag/AgCl electrodes, our values will be lower than resistances in zero Cl solutions. From Fig. 6 we cannot extrapolate to zero Cl, but in Cl-free solutions $\psi_{SM} = 147$ mV (Frömter *et al.*, 1974; Slegers *et al.*, 1975). From Fig. 6 we read $R_{SM} \simeq 30\,\Omega cm^2$ when $\psi_{SM} = 147$ mV. This extrapolated value is still significantly different from $54\,\Omega cm^2$. Therefore, current pulses of 5 msec duration already lead to an overestimation of the epithelial resistance due to polarization. Table 3 also shows that calculated resistance values reported by Slegers *et al.* (1975) are overestimated by about one order of magnitude. Their calculations were based on the assumption that unidirectional Cl fluxes from serosa to mucosa would provide reliable estimates for the epithelial permeability to Cl ions. In general this is true only if the movement is restricted to an extracellular pathway and thus approximates flow across a single barrier (Schultz & Frizzell, 1976). In our study we have shown that Cl ions in the serosal fluid have only a small effect on the resistance,

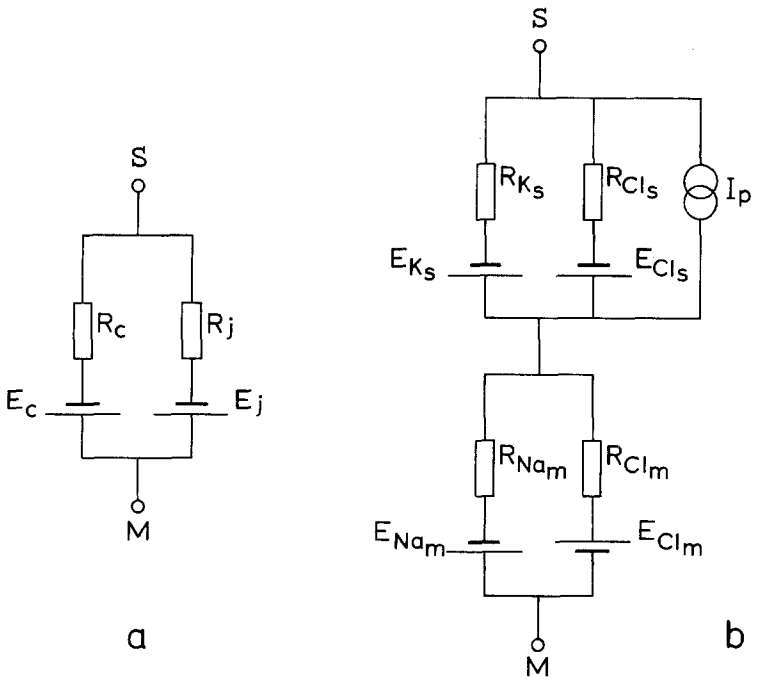

Fig. 10. Equivalent electrical circuits of rabbit salivary main duct epithelium; for explanation, *see* text

when ouabain is absent. This indicates that the active transport mechanism in the serosal membrane influences the serosa to mucosa flux of Cl, hence unidirectional Cl fluxes will lead to erroneous permeabilities.

We will discuss now in more detail whether the low resistance of ductal epithelium is due to extracellular shunting or to unusually high ionic permeabilities of the limiting cell membranes. In Fig. 10*a* an equivalent electrical circuit of an epithelium in its most simple form is given. E_c is here the sum of the EMF's generated across the cell membranes with a total resistance R_c. R_j indicates an extracellular shunt with a possible EMF that may arise in the presence of transepithelial ionic asymmetries. The transepithelial potential and resistance are now

$$\psi_{SM}=\frac{R_j E_c+R_c E_j}{R_j+R_c} \tag{1}$$

and

$$R_{SM}=\frac{R_c R_j}{R_c+R_j}. \tag{2}$$

When a variation in R_j determines the variation in R_{SM}, it is obvious that there will be a direct relationship between ψ_{SM} and R_{SM}. This is indeed the case for most epithelia (Augustus *et al.*, 1977). In SO_4 as well as in Cl-containing solutions we observed an inverse relationship between ψ_{SM} and R_{SM}. Similar inverse relationships have been reported for amphibian and mammalian urinary bladders with resistance three orders of magnitude greater than ductal epithelium (Higgins *et al.*, 1975; Lewis & Diamond, 1975). As pointed out by Higgins *et al.* (1975) this phenomenon can be explained when the rate of active Na transport is regulated by the Na conductance of the luminal membrane. Thus a lower resistance evokes a higher pumping rate and electrical potential across the contraluminal membrane. Therefore, the inverse relationship between ψ_{SM} and R_{SM} provides good evidence that the cellular route is the dominating conductor in the circuit. In support of this conclusion are the results of the experiments with ouabain, amiloride and temperature. From these experiments the conclusion was drawn that changes in intracellular concentrations of Na, K, and Cl affected the resistance of the cell membranes. Changes in the resistance of the cellular route are seen only if the shunt resistance is not too low. In most epithelia the overall resistance increases instead of decreasing after ouabain application. The increase is ascribed to a decrease in the width of the intracellular spaces, leading to an increased shunt resistance (Voute & Ussing, 1970; Smulders, Tormey & Wright, 1972). In the literature we found two epithelia where the resistance decreased after ouabain application, namely, piglet gastric mucosa (from 115 to 76 Ωcm^2; Forte *et al.*, 1975) and sheep rumen (from 1090 to 318 Ωcm^2; Herreira *et al.*, 1966). In addition, rabbit main duct responds asymmetrically to changes in Na, K, and Cl concentrations in the bathing solutions. Such an asymmetry is not compatible with the existence of a low extracellular shunt resistance. The values for apparent activation energies calculated for main duct resistance do not provide evidence for a cellular or paracellular route. The values are comparable to those reported for *aplysia* neurons, muscle, and rabbit jejunum (Bianchi, Giordane & Repetto, 1972).

In low Cl solutions we can estimate the ratio R_j/R_c as follows: using Eqs. (1) and (2) and assuming $E_j=0$, since the solutions are symmetrical, we find

$$R_c \cdot \psi_{SM} = R_{SM} \cdot E_c. \tag{3}$$

In 2 mM Cl symmetrically, $\psi_{SM}=120$ mV and $R_{SM}=26\ \Omega cm^2$. It is reasonable to assume $E_c<200$ mV; hence $120\text{ mV}<E_c<200$ mV. Using

Eqs. (1) and (3) we find $26 < R_c < 43\,\Omega\text{cm}^2$ and $\infty > R_j > 66\,\text{cm}^2$. The ratio R_j/R_c is then at least 1.5. In 100 mM Cl symmetrically $\psi_{SM} = 18\,\text{mV}$ and $R_{SM} = 12\,\Omega\text{cm}^2$. These changes can be brought about either by changes in R_j and E_c while R_c remains constant or by changes in R_c and E_c while R_j remains constant. The first explanation is highly unlikely in view of the evidence presented above. In the second situation we can calculate $12 < R_c < 15\,\Omega\text{cm}^2$ and $18 < E_c < 24\,\text{mV}$, while the ratio R_j/R_c will be at least 4.4.

In Fig. 10*b* an equivalent circuit is given for the cellular route across ductal epithelium. The minimum requirements are that the serosal membrane is permeable to K and Cl, and the luminal membrane to Na and Cl ions (Knauf, 1972*a*; Frömter *et al.*, 1974). In addition, a rheogenic pump can generate a current, I pump, across the lumped serosal membrane resistance, R_s (Augustus, 1976). For the transepithelial PD we can write:

$$\psi_{SM} = \frac{R_{\text{Cl}_s} \cdot E_{\text{K}} + R_{\text{K}} \cdot E_{\text{Cl}_s}}{R_{\text{Cl}_s} + R_{\text{K}}} + I\,\text{pump} \cdot R_s + \frac{R_{\text{Cl}_M} \cdot E_{\text{Na}} - R_{\text{Na}} \cdot E_{\text{Cl}_M}}{R_{\text{Cl}_M} + R_{\text{Na}}}. \quad (4)$$

In this circuit E_{K}, E_{Cl_s}, E_{Na}, and E_{Cl_M} are the equilibrium potentials for K and Cl ions across the serosal membrane and for Na and Cl ions across the luminal membrane, respectively. The serosal and mucosal membranes are most probably mosaics of K and Cl conductors and of Na and Cl conductors, respectively. The partial conductance for K will depend on the number and distribution of ions on either side and within the membrane. In analogy with the axonal membrane of the squid, for small K current we can write:

$$g_{\text{K}} = \frac{P_{\text{K}} F^3 E_s \text{K}_s}{R^2 T^2 [1 - \exp(-E_s F/RT)]} \quad (5)$$

(Hodgkin & Katz, 1949) where g_{K} is the partial K conductance, P_{K} is the K permeability, E_s is the serosal membrane PD, and K_s is the serosal K concentration. The other constants have the usual meaning. Similar equations can be written for g_{Na}, g_{Cl_s}, and g_{Cl_M} with the following changes in mind: the intracellular Na concentration and the PD across the mucosal membrane (E_M) appear in g_{Na}; the intracellular Cl concentration and E_s appear in g_{Cl_s}; and the luminal Cl concentration and E_M appear in g_{Cl_M}. Our resistance measurements indicate that a change in ionic composition on either side of the epithelium has more than one effect, and therefore interpretation of the observed change in ψ_{SM} be-

comes rather difficult. For example, increasing the luminal Cl concentration may have the following effects: (i) a change in E_{Cl_M}, (ii) a decrease in R_{Cl_M} which results in shunting of E_{Na}, and (iii) an increase in $[Cl]_c$ resulting in a change in E_{Cl_s} and a decrease in R_{Cl_s} which in turn results in shunting of E_K and of the potential generated by the pump. Another example is the relation between ψ_{SM} and the luminal Na concentration (Fig. 7) where a slope of 80 mV is observed between 55 and 144 mM Na. The main effect of increasing Na_M might be stimulation of I pump, since between 2 and 10 mM and after ouabain application there is no response at all of ψ_{SM} on increasing the Na concentration.

As a conclusion we may state that a change in ψ_{SM} after a change in the composition of the bathing fluids cannot be simply interpreted in terms of ionic selectivities of the cell membranes. There are several parameters in Eq. (4) which will change at the same time and exert their effect on the transepithelial PD. For a more quantitative description of the system it is imperative to follow the changes in the intracellular compartment by means of ion-selective micro-electrodes. Such experiments are currently in progress.

In rabbit main duct we observed an increase in resistance after addition of amiloride. Incubating the tissue in ouabain reduced the amiloride effect. We explained this amiloride effect by postulating that part of it was due to intracellular concentration changes. In other tight epithelia, like rabbit urinary bladder, a feedback mechanism was postulated in which cellular Na concentration regulates the Na conductance in the luminal membrane (Lewis *et al.*, 1976). If such a mechanism is present in rabbit main duct epithelium, then inhibition of the pump by ouabain leads to an increase in $[Na]_c$ which reduces the amiloride-sensitive conductance. This mechanism could explain why ouabain reduces the amiloride effect. However, in rabbit main duct we have found no evidence for a feedback mechanism. After ouabain application we observe a decrease in resistance even after 20 sec. If a feedback mechanism did exist, an increase in resistance should have been the result of ouabain addition as observed by Lewis *et al.* (1976).

One could raise the question whether the low resistance is due to surface area amplification caused by numerous folds or microvilli on the luminal surface. However, histological observations are against this explanation. Infoldings and microvilli are much less developed than those in small intestine or proximal tubules, which are epithelia with comparable resistances (Slegers *et al.*, 1975). Remarkable is the fact that the main duct permeability to small polar nonelectrolytes and water is

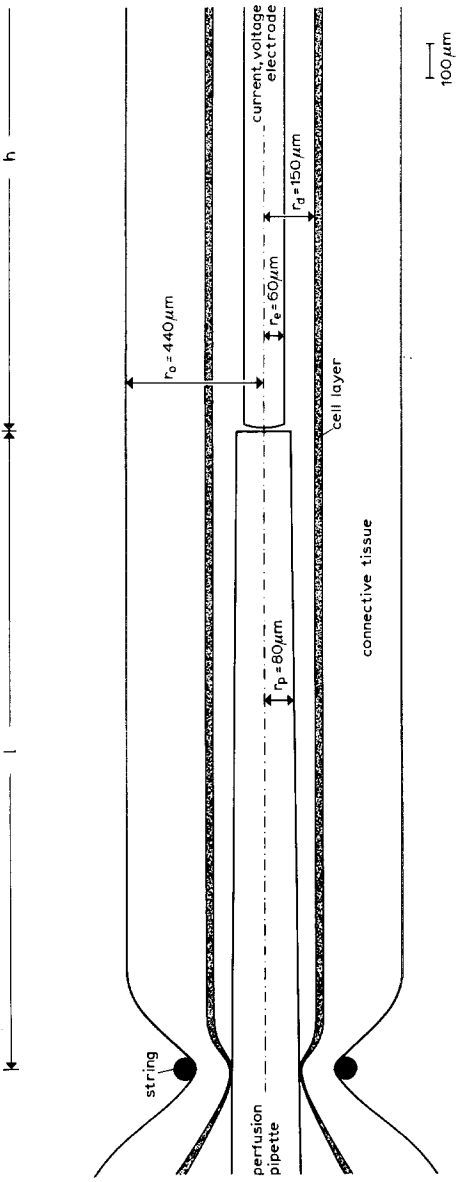

Fig. 11. Position of perfusion pipettes and axial electrode in the lumen of salivary main duct. For details and explanation *see* Appendix

not different from that of other tight epithelia (Augustus *et al.*, 1977). These findings indicate that ionic pathways through cell membranes are clearly distinctive from the overall permeability governed by the lipid composition of the membranes. By utilizing this principle, rabbit main duct epithelium is adapted very well to its task, reabsorbing NaCl from fast flowing saliva without reabsorbing water.

Appendix

The localization of the current electrode in the lumen of the main duct is sketched in Fig. 11. The axial electrode fills the space between the open ends of the perfusion pipettes (length h). The current field will be nonhomogeneous over a length l, the distance between the open end of the perfusion pipette and the string around the duct. Therefore, it is not correct to state: $R_{SP}=R\times h\Omega$cm, where

$$R=\frac{\delta V}{\delta I}(t\to 0) \quad \text{and} \quad R_{SP} \text{ specific resistance}$$

or

$$R_{SP}=R\cdot(h+2l)\,\Omega\text{cm}.$$

The real value for R_{SP} will be in between these two limits

$$R\cdot h<R_{SP}<R\cdot(h+2l).$$

In this appendix we derive an equation for R_{SP} in which corrections for this inhomogeneity and for resistances in the connective tissue and Ringer's solutions between the electrodes are introduced. A list of symbols to be used is given first:

R_i – specific resistance of the Ringer's solution in Ωcm
R_{SM} – specific resistance of the duct wall, Ωcm^2
r_e – radius of the luminal electrode, cm
r_p – radius of the perfusion pipettes, cm
r_d – radius of the duct lumen, cm
r_o – radius of the duct including the connective tissue layer, cm
h – the distance between the open ends of the perfusion pipettes, cm
l – the distance between the open end of a perfusion pipette and the string around the duct, cm
R – the measured resistance $\delta V/\delta I(t\to 0)$, Ω
R_l – the input resistance of duct length l, Ω
R_d – the resistance of the epithelium of length h, Ω
R_s – the resistance of the connective tissue plus fluid layers between the voltage electrodes over length h, Ω

The two parts of the main duct with length l are treated as one dimensional cables (with input resistance R_L) terminated by an infinite resistance. The part of the duct with length h has a resistance $R_h=R_d+R_s$. The measured resistance R is now equal to:

$$R=\frac{R_h \cdot R_{l/2}}{R_{h+} R_{l/2}}=\frac{(R_d+R_s)\cdot R_{l/2}}{R_d+R_s+R_{l/2}} \tag{A1}$$

or

$$\frac{1}{R}=\frac{1}{R_d+R_s}+\frac{2}{R_l} \tag{A2}$$

By definition:

$$R_d=\frac{r_{sm}}{h} \tag{A3}$$

and

$$r_{sm}=\frac{R_{SM}}{2\pi r_d} \quad \text{(Eisenberg \& Johnson, 1970)} \tag{A4}$$

where r_{sm} is the specific resistance of the epithelium in Ωcm. Also by definition:

$$R_S=\frac{R_i}{2\pi h}\ln\frac{r_0}{r_e} \quad \text{(Eisenberg \& Johnson, 1970)} \tag{A5}$$

$$R_l=\sqrt{r_{sm}\cdot r_i}\cdot \operatorname{cotgh}\left(l\cdot\sqrt{\frac{r_i}{r_{sm}}}\right) \quad \text{(Feldtheller, 1953)} \tag{A6}$$

and

$$r_i=\frac{R_i}{\pi(r_d^2-r_p^2)} \quad \text{(Eisenberg \& Johnson, 1970)} \tag{A7}$$

where r_i is the specific resistance of the Ringer's solution between the perfusion pipette and the cell layer (Ω/cm).

Substituting Eqs. (A3), (A5), and (A6) into Eq. (A2) gives us:

$$\frac{1}{R}=\frac{1}{\frac{r_{sm}}{h}+\frac{R_i}{2\pi h}\ln\frac{r_0}{r_e}}+\frac{2}{\sqrt{r_{sm}r_i}\cdot\operatorname{cotgh}\left(l\sqrt{\frac{r_i}{r_{sm}}}\right)}. \tag{A8}$$

Substituting

$$l\sqrt{\frac{r_i}{r_{sm}}}=\frac{x}{2} \tag{A8a}$$

and after rearranging we arrive at:

$$\frac{r_i}{R}=\frac{h}{\frac{(2l)^2}{x^2}+\frac{r_d^2-r_p^2}{2}\cdot\ln\frac{r_0}{r_e}}+\frac{2x}{2l}\operatorname{tgh}\frac{x}{2}. \tag{A9}$$

For $\operatorname{tgh}\frac{x}{2}$ we can write $\frac{1-e^{-x}}{1+e^{-x}}$, and for r_d, r_p, r_o, and r_e we can substitute 0.015 cm, 0.008 cm, 0.044 cm, and 0.006 cm, respectively (*see* Fig. 11):

$$\frac{r_i}{R}=\frac{h}{\frac{(2l)^2}{x^2}+1.6\times10^{-4}}+\frac{2x}{2l}\cdot\frac{1-e^{-x}}{1+e^{-x}}. \tag{A10}$$

From the measured h, $2l$, R, and r_i [Eq. (A7)], x can be calculated by means of an iterative procedure.

r_{sm} is then calculated from Eq. (A8*a*)

$$r_{sm}=\frac{(2l)^2 r_i}{x^2}.$$

After multiplying r_{sm} by $2\pi r_d$ we find R_{SM}, the specific resistance of ductal epithelium in Ωcm^2.

References

Augustus, J. 1976. Evidence for electrogenic sodium pumping in the ductal epithelium of rabbit salivary gland and its relationship with (Na+K)-ATPase. *Biochim. Biophys. Acta* **419**:63

Augustus, J., Bijman, J. 1977. An electrode which simultaneously passes current and measures potential, and is chloride selective. *Pfluegers Arch.* **370**:87

Augustus, J., Bijman, J., Os, C.H. van, Slegers, J.F.G. 1977. High conductance in an epithelial membrane not due to extracellular shunting. *Nature (London)* **268**:657

Augustus, J., Cuperus, P. 1977. A temperature control system for small biological preparations, displaying relatively fast heating and cooling capability. *IEEE Trans. Biomed. Eng.* **24**:291

Bentley, P.F. 1968. Amiloride: A potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (London)* **195**:317

Bianchi, A., Giordane, B., Repetto, F. 1972. Chloride transport in rabbit isolated jejunum and ileum. *Arch. Fisiol.* **69**:267

Biber, T.U.L. 1971. Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. Gen. Physiol.* **58**:131

Delcastillo, J., Machne, X. 1953. Effect of temperature on the passive electrical properties of the muscle fibre membrane. *J. Physiol. (London)* **120**:431

Eisenberg, R., Johnson, E.A. 1970. Three dimensional electrical field problems in physiology. *In:* Progress in Biophysics and Molecular Biology. Vol. 20, p. 5. J.A.V. Butler and D. Noble, editors. Pergamon Press, Oxford

Erlij, D. 1976. Basic electrical properties of tight epithelia determined with a simple method. *Pfluegers Arch.* **364**:91

Feldtheller, R. 1953. Einführung in die Vierpoltheorie der elektrischen Nachrichtentechnik. Hirzel, Stuttgart

Frizzell, R.A., Koch, M.J., Schultz, S.G. 1976. Ion transport by rabbit colon. I. Active and passive components. *J. Membrane Biol.* **27**:297

Frömter, E., Gebler, B., Schopow, K., Pockrandt-Hemstedt, H. 1974. Cation and anion permeability of rabbit submaxillary main duct. *In:* Alfred Benzon Symposion VII, Secretory Mechanisms of Exocrine Glands. N.A. Thorn and O.H. Petersen, editors, p. 496. Munksgaard, Copenhagen

Higgins, J.T., Cesaro, L., Gebler, B., Frömter, E. 1975. Electrical properties of amphibian urinary bladder epithelia. *Pfluegers Arch.* **358**:41

Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**:37

Knauf, H. 1972*a*. The isolated salivary duct as a model for electrolyte transport studies. *Pfluegers Arch.* **333**:82

Knauf, H. 1972*b*. The minimum requirements for the maintenance of active sodium transport across the isolated salivary duct epithelium of the rabbit. *Pfluegers Arch.* **333**:326

Lewis, S.A., Diamond, J.M. 1975. Active sodium transport by mammalian urinary bladder. *Nature (London)* **253**:747

Lewis, S.A., Eaton, D.C., Diamond, J.M. 1976. The mechanism of Na^+ transport by rabbit urinary bladder. *J. Membrane Biol.* **28**:41

Marchiafova, P. 1970. The effect of temperature change on membrane potential and conductance in aphysia giant nerve cell. *Comp. Biochem. Physiol.* **34**:847

Martin, C.J., Frömter, E., Gebler, B., Knauf, H., Young, J.A. 1972. Electrolyte transport in the excurrent duct system of the submaxillary gland II. *In:* Oral Physiology. N. Emmelin and Y. Zotterman, editors. p. 115. Pergamon Press, Oxford

Nagel, W., Dörge, A. 1970. Effect of amiloride on sodium transport of frog skin. I. Action on intracellular sodium content. *Pfluegers Arch.* **317**:84

Schultz, S.G. 1972. Electrical potential differences and electromotive forces in epithelial tissues. *J. Gen. Physiol.* **59**:794

Schultz, S.G., Frizzell, R.A. 1976. Ionic permeability of epithelial tissues. *Biochim. Biophys. Acta* **443**:181

Slegers, J.F.G., Moons, W.M., Stadhouders, A.M., Idzerda, P.P. 1975. The contribution of a chloride shunt to the transmucosal potential of the rabbit submaxillary duct. *J. Membrane Biol.* **25**:213

Smulders, A.P., Tormey, J.McD., Wright, E.M. 1972. The effect of osmotically induced water flows on the permeability and ultrastructure of the rabbit gallbladder. *J. Membrane Biol.* **7**:164

Voute, C.J., Ussing, H.H. 1970. Quantitative relation between hydrostatic pressure gradient, extracellular volume and active sodium transport in the epithelium of frog skin. *Exp. Cell Res.* **62**:375