LETTER TO THE EDITOR

Impalement Artifacts in Microelectrode Recordings of Epithelial Membrane Potentials

Dear Sir:

Reuss and Finn recently reported that the serosal membrane potential (V_i) of toad bladder epithelium responds almost instantaneously to changes of the mucosal membrane potential (V_o). In their experiments a microelectrode advanced through the mucosal cellular border was used to record both V_o and V_i while the composition of the mucosal solution was varied. V_i was observed to change in the same direction as V_o and with a delay of only 10 ms. Paracellular shunting cannot explain this phenomenon, which was attributed to an unknown mechanism (Reuss and Finn, 1975).

A convincing demonstration of such coupling mechanisms would be of great interest. I should like to point out, however, that the reported observation may well be an artifact. Shunting of the mucosal membrane at the point of impalement can be expected to permit flow of excess current which passes through the serosal membrane where V_i is recorded. Current source is the unimpaled adjacent tissue. The excess current will change with transepithelial voltage V and thus cause apparent variations of V_i at the point where the microelectrode recording is done. The apparent variations will occur rapidly and be of the same sign as the variations of V. In order to predict the magnitude of such impalement-artifacts some quantitative relationships will be derived. Let us consider the equivalent circuit of Fig. 1. On the left, the battery voltages U_o and U_i represent the open circuit membrane potentials of the mucosal and serosal membrane, while R_o and R_i are the corresponding membrane resistances (ohm · centimeter^2). R_p is a paracellular shunt resistance, which also passes shunt current due to edge damage. The total current (I) passed through the epithelium splits up in paracellular and cellular components (I_p and I_c, microamperes per centimeter^2), while the transepithelial voltage V is the sum of V_o and V_i.

Since the voltage ($I_p \cdot R_p$) across the paracellular pathway is identical with that across the cellular pathway

$$U_o + U_i + (I - I_p)(R_o + R_i) - I_p R_p = 0.$$

Rearrangement and substitution with $I_c = I - I_p$ yields an expression for the current through the cellular pathway:

$$I_c = [I \cdot R_p - (U_o + U_i)]/(R_o + R_i + R_p). \tag{1}$$

The voltages across serosal and mucosal membranes are derived from

$$V_i = U_i + I_c \cdot R_i, \quad V_o = U_o + I_c \cdot R_o$$

to be

$$V_i = \frac{U_i(R_o + R_p) - U_o R_i}{R_o + R_i + R_p} + \frac{I \cdot R_i \cdot R_p}{R_o + R_i + R_p}, \tag{2}$$

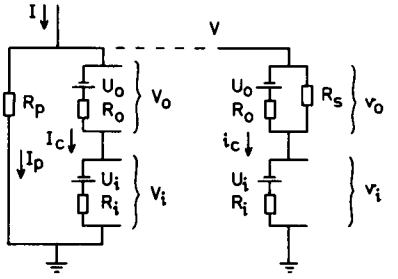

FIGURE 1 Equivalent circuit of epithelial cells. R_p represents the resistance of a paracellular shunt pathway and R_s (to the right) the resistance of a shunt produced by the microelectrode in the mucosal membrane of the impaled cell. The serosal (inner) solution is grounded. Inward current (arrows) is taken positive. Therefore, membrane voltages (U, V, and v) are taken positive when the outer (or cellular) potential is positive with respect to the cellular (or inner) potential.

$$V_o = \frac{U_o(R_i + R_p) - U_iR_o}{R_o + R_i + R_p} + \frac{I \cdot R_o \cdot R_p}{R_o + R_i + R_p}, \tag{3}$$

while the total voltage is

$$V = \frac{R_p(U_o + U_i)}{R_o + R_i + R_p} + \frac{I \cdot R_p(R_o + R_i)}{R_o + R_i + R_p}. \tag{4}$$

Suppose a microelectrode impales the mucosal membrane of one cell. If the membrane does not seal perfectly around the electrode, a shunt resistance R_s ($\Omega \cdot cm^2$) has been placed in parallel to R_o of this cell as shown in the right part of Fig. 1. The potentials of the mucosal and serosal membranes of this cell will be called v_o and v_i. Their sum will always be V since the cell is "voltage clamped" by the surrounding undamaged epithelium, which constitutes a low impedance current source. Therefore,

$$V = v_o + v_i = U_i + U_o \frac{R_s}{R_o + R_s} + i_c\left[R_i + \left(\frac{1}{R_o} + \frac{1}{R_s}\right)^{-1}\right],$$

where i_c is the current (per unit area) flowing through the impaled cell. Rearrangement yields

$$i_c = \frac{V - U_i - U_oR_s/(R_o + R_s)}{R_i + [(1/R_o) + (1/R_s)]^{-1}}. \tag{5}$$

The membrane potentials v_o and v_i are given by

$$v_i = \frac{U_iR_o - U_oR_i + V \cdot R_i(R_o + R_s)/R_s}{R_o + R_i + R_oR_i/R_s}, \tag{6}$$

$$v_o = \frac{U_oR_i - U_iR_o + V \cdot R_o}{R_o + R_i + R_oR_i/R_s}. \tag{7}$$

As they stand, these equations are useful for cases where the whole epithelium is voltage clamped since then V is the command voltage. For an analysis of the current clamp situation we substitute V from Eq. 4 into Eqs. 6 and 7 and obtain

$$v_i = \frac{U_i R_o R_s - U_o R_i R_s}{R_o R_s + R_i R_s + R_o R_i} + \frac{(U_o + U_i) R_p R_i (R_o + R_s) + I R_p R_i (R_o + R_i)(R_o + R_s)}{(R_o R_s + R_i R_s + R_o R_i)(R_o + R_i + R_p)}, \quad (8)$$

$$v_o = \frac{U_o R_i R_s - U_i R_o R_s}{R_o R_s + R_i R_s + R_o R_i} + \frac{(U_o + U_i) R_o R_s R_p + I R_o R_s R_p (R_o + R_i)}{(R_o R_s + R_i R_s + R_o R_i)(R_o + R_i + R_p)}. \quad (9)$$

When recordings are done in the current clamp mode and small current pulses of amplitude ΔI are passed through the epithelium, as in the experiments by Reuss and Finn, the deflections of v_i and v_o can be seen from Eqs. 8 and 9. For instance

$$\Delta v_i = \Delta I \frac{R_p R_i (R_o + R_i)(R_o + R_s)}{(R_o + R_i + R_p)(R_o R_s + R_i R_s + R_o R_i)}. \quad (10)$$

These relationships can be used to estimate the error caused by the impalement-shunt in a determination of R_o and R_i from microelectrode recordings. Let us assume for simplicity that the paracellular shunt is negligible ($R_p = \infty$). When in this situation the deflections of Δv_o and Δv_i divided by the current density ΔI are taken to indicate R_o and R_i, R_o will be underestimated and R_i will be overestimated by the term

$$R_i R_o^2 / (R_o R_i + R_o R_s + R_i R_s).$$

Thus the ratio $\Delta v_o / \Delta v_i$ is not equal to R_o / R_i, as assumed by Reuss and Finn, but smaller:

$$\Delta v_o / \Delta v_i = (R_o / R_i)[R_s / (R_o + R_s)],$$

and this is also true when $R_p < \infty$.

When no external current is passed ($I = 0$), v_i is still a function of U_i, U_o, and all resistances:

$$v_i = \frac{U_i [R_o R_s (R_o + R_i) + R_p (R_o R_s + R_i R_s + R_o R_i)] + U_o [R_o R_i R_p - R_i R_s (R_o + R_i)]}{(R_o R_s + R_i R_s + R_o R_i)(R_o + R_i + R_p)}. \quad (11)$$

In Eq. 11, v_i becomes equal to V_i (Eq. 2), when the shunt resistance R_s introduced by the microelectrode is large enough to be without effect:

$$v_i = V_i = [U_i (R_o + R_p) - U_o R_i] / (R_o + R_i + R_p). \quad (2A)$$

Thus the difference between V_i (Eq. 2 for $I = 0$) and v_i (Eq. 11) is an impalement artifact no-

ticeable in the potential of the *serosal* membrane. How v_i varies with U_o depends on the magnitude of R_p. When R_p is small, v_i tends to decrease with increasing U_o. When R_p is very large, Eq. 11 simplifies to

$$v_i = U_i + U_o\left[1 + \frac{R_s}{R_o} + \frac{R_s}{R_i}\right]^{-1}. \qquad (11\,A)$$

Then v_i changes in the same direction as U_o, as observed by Reuss and Finn. For instance, when the Na-concentration $(Na)_o$ of the mucosal solution is suddenly increased, U_o will become more negative and v_i will also become more negative. The change of v_i will be somewhat smaller if R_o decreases at the same time. Nevertheless, with $R_i = 0.3$ and $R_s = 0.38$ k$\Omega \cdot$ cm^2 a change of U_o from 0 to -50 mV and simultaneously of R_o from 10 to 1 k$\Omega \cdot$ cm^2 would make v_i more negative by 19 mV. At the same time $\Delta v_o/\Delta v_i$ becomes smaller than R_o/R_i by a factor 0.28. With $R_s = 0.92$ k$\Omega \cdot$ cm^2 v_i becomes more negative by 10 mV and $\Delta v_o/\Delta v_i$ becomes smaller than R_o/R_i by a factor of 0.48.

It is interesting to realize that a shunt resistance as low as 380 $\Omega \cdot$cm^2 (780 MΩ in an apical cell membrane of 7 $\times$ 7 μm area) can be caused by a channel filled with Ringer's solution (100 $\Omega \cdot$ cm) of 100 Å in length and only 32 $\times$ 32 Å in area. An impalement shunt channel of similarly small dimensions may often if not always be established by microelectrode tips of 5,000 Å in diameter. It will become noticeable only when the punctured cell is small and of large membrane resistance (erythrocyte) or when an unimpaled series membrane permits detection of the excess current flowing through the impalement shunt (epithelial cell).

The equations derived above have many implications not all of which can be discussed here. The reader will have noted that such processes as active transport, degradation of diffusion gradients through the shunt conductance $(1/R_s)$ or lateral cellular coupling and uncoupling as well as charging of membrane capacitances have not been considered in the derivation. Nevertheless, the equations demonstrate quite clearly that a shunt produced by the microelectrode in the mucosal membrane can explain the reported change of the serosal membrane potential in response to a change of the mucosal membrane potential.

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REFERENCES

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