J. Membrane Biol. 59, 173–177 (1981)

The Journal of
Membrane Biology

The Conventional Short-Circuiting Technique Under-Short-Circuits Most Epithelia

Y.-H. Tai and C.-Y. Tai

Department of Gastroenterology, Walter Reed Army Institute of Research, Washington, D.C. 20012, and
Department of Physiology, George Washington University School of Medicine, Washington, D.C. 20037

Summary. The relationships among ion current, membrane potential difference, and resistance of an epithelium are studied. The short-circuit technique introduced by Ussing and Zerahn does not completely short circuit the epithelium if the series resistance parallel to the cell layer between the voltage electrodes is not properly compensated. The residual potential difference across the epithelial cell layer in the "short-circuit state" is proportional to both the measured short-circuit current and the resistance of the diffusion barriers not compensated. In the conventionally short-circuited small intestinal mucosa the villus and crypt areas are hypo-polarized to different degrees rather than simultaneously hyper- and hypo-polarized. Short-circuiting the whole tissue reduces but does not abolish the passive net ion movement across the tissue. Measurements of the electrical properties of the whole and denuded rat distal small intestine in HCO_3-Ringer solution containing 10 mM glucose reveal that the measured short-circuit current has under-estimated approximately 33% of the true short-circuit current and that the passive net Na flux from serosa to mucosa and Cl flux from mucosa to serosa are not negligible in the "short-circuit state."

The short-circuit technique introduced by Ussing and Zerahn [8] in their studies of ion transport across isolated frog skin has contributed greatly to the investigation of ion transport across epithelial membranes. The short-circuit conditions are accomplished by bathing both sides of the tissue with identical solutions in the absence of hydrostatic pressure difference and at the same time adjusting the external current so that the electrical potential difference (PD) across the tissue is zero. Under these conditions, the electrochemical potential of any ion is the same in both solutions and there should be no net movement across the tissue resulting from diffusion alone. The finding of a net ion flux in the short-circuit state indicates either that the ion is subject to forces arising from its interaction with other fluxes or that the net flux is a result of "active transport." The short-circuit current, in principle, represents the algebraic sum of the net fluxes of all ions whose flows across the tissue cannot be attributed solely to differences in electrochemical potential.

In short-circuiting a cell layer, the resistance of the solutions, connective tissue, and muscle layers in series with the cell layer between the voltage electrodes should be taken into consideration. The true short-circuit current should be the short-circuit current corrected for the series resistance. In the study of current-voltage relations in the membrane of the giant axon of *Loligo*, Hodgkin, Huxley, and Katz [4] were able to estimate the series resistance either by dividing the time constant determining the decline of the capacitative curve by the measured value of the membrane capacity or by the change of the potential difference elicited by a rectangular current pulse, as a function of time. In the field of epithelial transport, only the solution resistance has been compensated either manually or automatically [1, 2]. Recently, Gebhardt and Nell [3] were able to measure the series resistance using a method similar to the second method of Hodgkin, Huxley, and Katz [4] by analyzing the voltage deflection elicited by a rectangular current registered on a storage oscilloscope.

With a different view, Rehm concluded from an equivalent circuit model that the whole *in vitro* tissue rather than the epithelial cell layer should be short-circuited [5]. In this paper, we like to show a simple analytical relationship between the true short-circuit current and the short-circuit current

0022-2631/81/0059-0173 $01.00

without compensating the resistance of the subepithelial tissue.

We also like to show that the villus and crypt areas are hypo-polarized to different degrees rather than simultaneously hyper- and hypo-polarized[1] in the conventionally short-circuited intestinal mucosa.

Finally, the true short-circuit current in the rat distal small intestine is estimated from the conventionally measured short-circuit current across the whole tissue and the resistance of the subepithelial tissue prepared from adjacent segment.

Relationship between Net Ion Fluxes and PD under Open-Circuit Conditions

Consider an epithelial tissue containing three layers, α, β, and γ in series, separating two compartments I and II (Fig. 1). α represents the epithelial cell layer, and β and γ represent the subepithelial and an unstirred layer, respectively. Identical bathing solutions are well stirred in compartments I and II. The PD of the tissue, V_{AB}, is measured between points A and B with a high impedance voltmeter. The resistances of α, β, and γ are R_α, R_β, and R_γ, respectively. The resistances of the bulk solution between A and γ and between B and β are R_A and R_B, respectively. I_a is the total ion current, resulting from the net ion fluxes directly or indirectly generated by the "pump(s)" in the epithelial cell layer. When the pump starts operating, V_{AB} is built up which in turn produces the diffusion current I_d to counteract the I_a. Since α is the ion current source, it exerts no resistance to I_a. The relationship between V_{AB} and ion currents, at any time, can be expressed as

$$V_{AB}=(I_d-I_a)(R_\beta+R_\gamma+R_A+R_B)+I_dR_\alpha \tag{1}$$

in which I_d-I_a is the net current across the layers. When a steady state is reached in which there is no net ion current flowing across the layers, i.e., $I_d=I_a$, then

$$V_{AB}=I_dR_\alpha=I_aR_\alpha. \tag{2}$$

Equation (2) indicates that V_{AB} in the steady state is determined by I_a and only the resistance of the epithelial cell layer, R_α.

In the steady state under open-circuit conditions

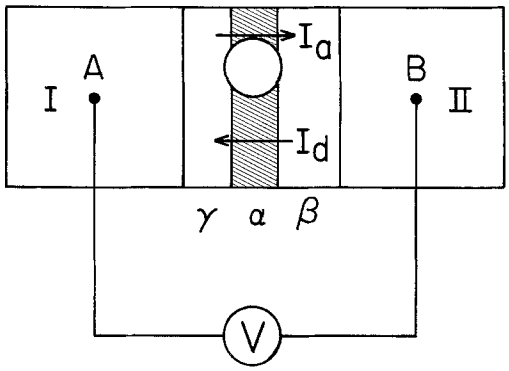

Fig. 1. An epthelial tissue containing three layers, α, β, and γ, separates two compartments I and II. The layer α is the epithelial cell layer, β and γ are the subepithelial layer and an unstirred layer, respectively. I_a is the ion current generated by the "pump" in α and I_d is the diffusional ion current driven by the electrical potential difference across the tissue, V_{AB}

the net passive ionic flow of ion i driven by V_{AB} can be given as

$$J_i=t_iI_a/z_iF \tag{3}$$

in which t_i is the transport number of ion i and defined as the fraction of the current I_d carried by the ion species i, z_i is the charge of the ion i and F is the Faraday constant. The direction of J_i is determined by the signs of the ion species and V_{AB}. If I_a represents a Na^+ current, the Na^+ concentration in the layer β would be increased. In the steady state, the diffusional Na^+ flux ($t_{Na}I_a/F$) from β to compartment I can be described by the Goldman-Hodgkin-Katz equation and the Na^+ flux across the β layer into compartment II is accompanied by an equal anion flux driven by the PD from compartment I to compartment II and is described by the Fick's first law of diffusion since the PD across β is zero. In the situation that I_a is resulted from a Cl^- current from β to γ, the Cl^- concentration in β would be decreased. In the steady state, the passive Cl^- flux from γ to β ($t_{Cl}I_a/F$) is also described by the Goldman-Hodgkin-Katz equation and the Na^+ flux driven by the PD from compartment II to compartment I is equal to the difference between the two Cl^- fluxes. If I_a is a combination of Na^+ and Cl^- currents, the net result of I_a on the passive ion movement would be the algebraic sum of the effect of each component of I_a.

Relationship between Net Ion Fluxes and Short-Circuit Current

When an external current I_e is applied in the same direction as I_a from D to C as shown in Fig. 2, we get

[1] This possibility was introduced and discussed by Drs. R.A. Frizzell, S.G. Schultz, M. Field, and D.W. Powell during the meetings of the Federation of American Societies for Experimental Biology in Dallas, Tex., 6–10 April 1979. The hypothetical effect of the simultaneous hyper- and hypo-polarization on ion transport across the short-circuited intestinal mucosa of the rat ileum was discussed by Tai and Decker [7].

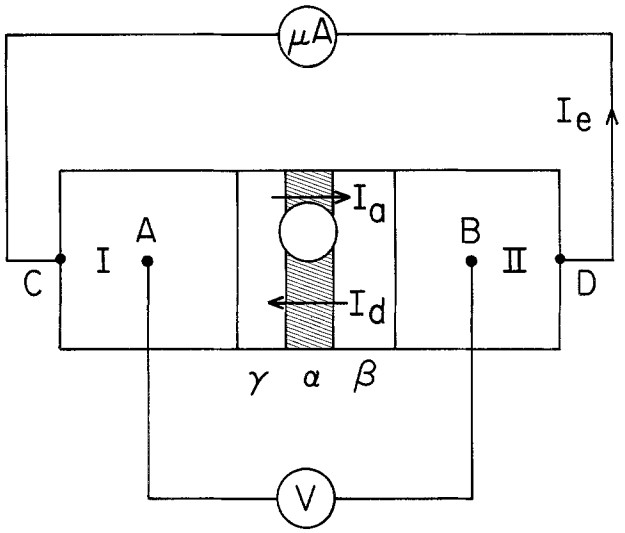

Fig. 2. The external current I_e is passed from D to C in order to vary V_{AB}

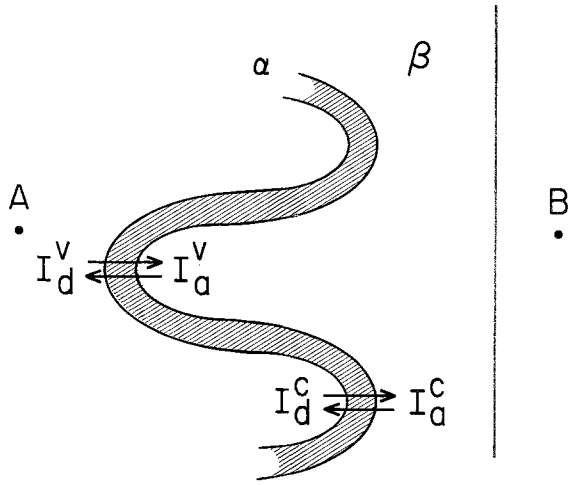

Fig. 3. α and β are the epithelial cell layer and the subepithelial layer of a section of the small intestine, respectively. The villus and crypt areas are designated by the superscripts v and c, respectively. The direction of I_a is from left to right and the direction of I_d from right to left

$$V_{AB}=(I_a-I_e)R_\alpha-I_e(R_\beta+R_\gamma+R_A+R_B). \tag{4}$$

Since the resistances R_A and R_B can be premeasured, a device is attached externally so that whatever I_e is passed through in the circuit, a PD of $I_e(R_A+R_B)$ is subtracted from the reading of V_{AB}. Equation (4) then becomes

$$V_{AB}=I_aR_\alpha-I_e(R_\alpha+R_\beta+R_\gamma). \tag{5}$$

If I_e is so varied that $V_{AB}=0$, then

$$[I_e]_{V_{AB}=0}\equiv I_e^0=I_aR_\alpha/(R_\alpha+R_\beta+R_\gamma). \tag{6}$$

I_e^o represents the measured short-circuit current and under-estimates the total net ion flow, I_a, or true short-circuit current by a factor of $R_\alpha/(R_\alpha+R_\beta+R_\gamma)$. Only when $R_\alpha\gg(R_\beta+R_\gamma)$, then I_e^o approaches I_a. Under these short-circuit conditions, the PD across α is not zero and can be given as

$$V_\alpha=(I_a-I_e^o)R_\alpha. \tag{7}$$

Substituting Eq. (6) into Eq. (7), we get

$$V_\alpha=I_e^o(R_\beta+R_\gamma). \tag{8}$$

Equation (8) indicates that the residual PD across α in the "short-circuit state" is proportional to both the measured short-circuit current and the sum of R_β and R_γ. Again, only when I_e^o or $(R_\beta+R_\gamma)$ approach zero then V_α becomes negligible.

If the layer γ represents an unstirred layer, the difference between R_γ and the resistance of a well-stirred solution with identical thickness is negligible (*see* Appendix). Then R_γ can be included in R_A. Equation (8) becomes

$$V_\alpha=I_e^oR_\beta. \tag{9}$$

The diffusion current, I_d, in this short-circuit state is

$$[I_d]_{V_{AB}=0}\equiv I_d^o=I_a-I_e^o=I_aR_\beta/(R_\alpha+R_\beta). \tag{10}$$

The passive ionic flow of i across the layer α is

$$J_i=t_iI_d/z_iF=t_iI_aR_\beta/(R_\alpha+R_\beta)z_iF. \tag{11}$$

Comparison between Eqs. (3) and (11) indicates that these passive net ionic flows are reduced but not abolished in the "short-circuit state."

Short-Circuiting the Small Intestine

The mammalian small intestine contains numerous parallel conductive pathways (shunt pathways) throughout the villus and crypt areas. Figure 3 shows a diagram of a section of small intestine including a villus and a crypt area. The net ion current generated by the epithelial cells and the diffusional ion current in each area are designated by a superscript v for villus and c for crypt. Since all the conductive pathways are parallel, the open-circuit PD in each pathway measured between points A and B should be identical in the steady state. As is similar to Eq. (2), we get

$$V_{AB}=I_a^vR_\alpha^v=I_a^cR_\alpha^c \tag{12}$$

in which R_α^v and R_α^c are the resistance of the villus and crypt area of the epithelial cell layer, respectively. Rearranging Eq. (12) gives

$$I_a^v/I_a^c=R_\alpha^c/R_\alpha^v. \tag{13}$$

The total ion current, I_a, and the resistance of the epithelial cell layer are

$$I_a = \sum_i I_a^i \tag{14}$$

and

$$R_\alpha = [\sum_i (R_\alpha^i)^{-1}]^{-1}. \tag{15}$$

The PD across the tissue can also be expressed as

$$V_{AB} = I_a R_\alpha. \tag{16}$$

When the external current, I_e^o, is applied to nullify the V_{AB}, similar treatment for getting Eq. (6) leads to

$$I_e^o = I_a R_\alpha / (R_\alpha + R_\beta) \tag{17}$$

in which $R_\beta = [\sum_i (R_\beta^i)^{-1}]^{-1}$. Equation (17) indicates, similar to Eq. (6), that the measured short-circuit current under-estimates the total ion current I_a. The distribution of I_e^o in all the pathways is

$$I_e^o = \sum_i I_e^{oi} \tag{18}$$

$$I_e^{oi} = I_a^i R_\alpha^i / (R_\alpha^i + R_\beta^i). \tag{19}$$

The superscript i indicates the i-th pathway. The residual PD across the epithelial cell layer in each area is given by

$$V_\alpha^i = I_e^{oi} R_\beta^i. \tag{20}$$

The ratio of V_α between a villus and a crypt area is

$$\frac{V_\alpha^v}{V_\alpha^c} = \frac{I_a^v R_\alpha^v R_\beta^v}{I_a^c R_\alpha^c R_\beta^c} \cdot \frac{R_\alpha^c + R_\beta^c}{R_\alpha^v + R_\beta^v}$$
$$= (R_\beta^v R_\alpha^c + R_\beta^v R_\beta^c)/(R_\beta^c R_\alpha^v + R_\beta^c R_\beta^v). \tag{21}$$

If $R_\alpha^c \geqq R_\alpha^v$, then

$$V_\alpha^v > V_\alpha^c \tag{22}$$

since $R_\beta^v > R_\beta^c$. The incomplete short-circuiting is probably more significant in the villus area than in the crypt area.

Recently, a technique of removing the epithelial cell layer in the rat jejunum was developed [6]. The epithelial cells from both the villus and crypt areas were completely removed but the structure of the subepithelial tissue was well preserved and the villiform organization was maintained. An electronmicrograph of the denuded tissue suggested the basement membrane remained intact when the epithelium was removed. The electrical properties of the subepithelial tissue prepared by the same technique and of the adjacent whole tissue in HCO_3-Ringer solution containing 10 mM glucose are shown in Table 1. The membrane PD is serosa positive and the short-circuit current represents a positive current from mucosa to serosa. The solution resistance between the tips of the agar bridges to the voltage electrodes was corrected by an automatic voltage clamp in obtaining the I_e^o for the whole tissue. The difference in resistance between the whole tissue and denuded tissue provides an estimate of the resistance of the epithelial cell layer, 21.4 Ω-cm^2 which is in good agreement with the value of 17.4 Ω-cm^2 found by Gebhardt and Nell [3]. The estimated true short-circuit current by Eq. (17) is 5.11 μeq/hr/cm^2. The residual PD across the epithelial cell layer, V_α, is estimated to be 1.0 mV, serosa positive. It is of importance to note that under these "short-circuit conditions" net passive Na flux from serosa to mucosa and Cl flux from mucosa to serosa are present and cannot be ignored; approximately 0.76 μeq/hr/cm^2 for Na flux assuming $t_{Na} = 0.45$ and 0.60 μeq/hr/cm^2 for Cl flux assuming $t_{Cl} = 0.35$. These estimated passive net fluxes of Na and Cl are approximately 40% of the net fluxes of Na and Cl measured under identical conditions [7]. When the relationship between short-circuit current and ion fluxes is evaluated, these corrections should be considered.

Finally, the analysis in this paper indicates that to strip off the serosal and muscle layers of small intestine in studies of ion transport in Ussing chambers is justified.

Table 1. Electrical properties of the whole tissue and subepithelial tissue of the rat distal small intestine in HCO_3-Ringer solution containing 10 mM glucose

	I_e^o	PD	G	R
Whole tissue	3.43 ± 0.51	2.94 ± 0.31	31.6 ± 1.3	31.9 ± 1.3
Subepithelial			96.5 ± 5.7	10.5 ± 0.6

The values are expressed as mean ± SE of four observations. The units are μeq/hr/cm^2 for I_e^o, mV for PD, Ω·cm^2 for R, and mmho/cm^2 for G which is the tissue conductance. The I_e^o and PD of the subepithelial tissue are virtually zero. The conductance (G) is determined as the relative change in current with respect to the change of 2 mV (±1.0 mV from zero) in PD. The sign of PD is serosal side positive with respect to the mucosal side.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

Appendix

The difference in resistance between two similar solutions depends on the differences in ion composition. If a tissue is bathed in a solution containing 140 mM NaCl and a steady-state rate of net transport of Na and Cl is reached, the change in Na or Cl concentration in the unstirred layer can be estimated by Fick's first law of diffusion, i.e.

$$J_i = -D_i(dC_i/dx) \tag{A1}$$

in which J_i, D_i, and dC_i/dx are the steady-state net flux, diffusion coefficient, and concentration gradient of ion i in the unstirred layer, respectively. Equation (A1) assumes that the effect of electrical potential gradient in the unstirred layer is negligible in comparison to the concentration gradient and that the water flows in response to the salt transport do not significantly change the salt concentration in the unstirred layer. Since the diffusion coefficient can be treated as a constant in a small concentration range, integration of Eq. (A1) over the thickness of the unstirred layer gives

$$\Delta C_i = -J_i \Delta x/D_i \tag{A2}$$

in which ΔC_i is the concentration difference of ion i across the unstirred layer and Δx is the thickness of the unstirred layer. Assuming $J_{Na} = 2\ \mu eq/hr/cm^2$, $D_{Na} = 0.04\ cm^2/hr$, and $\Delta x = 0.03$ cm, we get $\Delta C_{Na} = -1.5$ mM. The average decrease in Na concentration of the unstirred layer is about one half of the concentration difference across the unstirred layer. Hence, it is reasonable to assume that the difference in resistance between unstirred layer and bulk solution with identical thickness is negligible.

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Received 9 June 1980; revised 28 October 1980