cAMP Activation of Apical Membrane Cl\(^{-}\) Channels: Theoretical Considerations for Impedance Analysis

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ABSTRACT Transepithelial electrical impedance analysis provides a sensitive method to evaluate the conductances and capacitances of apical and basolateral plasma membranes of epithelial cells. Impedance analysis is complicated, due not only to the anatomical arrangement of the cells and their paracellular shunt pathways, but also in particular to the existence of audio frequency-dependent capacitances or dispersions. In this paper we explore implications and consequences of anatomically related Maxwell-Wagner and Cole-Cole dielectric dispersions that impose limitations, approximations, and pitfalls of impedance analysis when tissues are studied under widely ranging spontaneous rates of transport, and in particular when apical membrane sodium and chloride channels are activated by adenosine 3',5'-cyclic monophosphate (cAMP) in A6 epithelia. We develop the thesis that capacitive relaxation processes of any origin lead not only to dependence on frequency of the impedance locus, but also to the appearance of depressed semicircles in Nyquist transepithelial impedance plots, regardless of the tightness or leakiness of the paracellular shunt pathways. Frequency dependence of capacitance precludes analysis of data in traditional ways, where capacitance is assumed constant, and is especially important when apical and/or basolateral membranes exhibit one or more dielectric dispersions.

INTRODUCTION

A variety of hormones including aldosterone and insulin selectively stimulate Na\(^{+}\) transport in cell-cultured A6 epithelia by increase of the density of functional amiloride-sensitive epithelial Na\(^{+}\) channels (ENaCs) at the apical membranes of the cells (Blazer-Yost et al., 1996; Baxendale-Cox et al., 1997; Helman et al., 1998). In contrast, hormonal activation of Na\(^{+}\) transport mediated by the second messenger adenosine 3',5'-cyclic monophosphate (cAMP) is accompanied by stimulation of Cl\(^{-}\) transport (Yanase and Handler, 1986; Chalfant et al., 1993) involving activation of apical membrane Cl\(^{-}\) channels (Marunaka and Eaton, 1990; Nakahari and Marunaka, 1995; Kokko et al., 1997; Matsumoto et al., 1997). During the course of our studies aimed at understanding the interrelationships among hormones in regulation of Na\(^{+}\) transport it became necessary to know not only the magnitude of change of the apical membrane cAMP-activated chloride conductance, but also the consequences of activation of the chloride conductance in electrophysiological studies of Na\(^{+}\) transport, specifically in the A6 epithelium and more generally in other tissues where cAMP activates Na\(^{+}\) and Cl\(^{-}\) channels.

To address such questions we turned to impedance analysis of the A6 epithelium and found, as in frog skin (Awayda et al., 1999) that the analysis of A6 epithelia was more complicated due principally to audio frequency dielectric relaxation processes or dispersions (Liu and Helman, 1998). In this paper we have used data derived from our studies of A6 epithelia (Păunescu and Helman, 2001) to discuss and illustrate the guiding principles used in experimental design, methods of analysis, and interpretation of data. Although the emphasis of our original inquiry was to understand the role of cAMP in regulation of apical membrane Na\(^{+}\) and Cl\(^{-}\) channels using methods of impedance analysis, the scope of this project required a rigorous examination of the underlying theoretical considerations and principles that have not appeared in the epithelial transport literature. In this manuscript we emphasize the relevant considerations and principles appropriate to epithelia. The experimental findings presented in a companion paper (Păunescu and Helman, 2001) underscore the need for our theoretical considerations in understanding the mechanisms of hormonal action that activate not only Na\(^{+}\), but also apical membrane Cl\(^{-}\) channels in A6 epithelia.

Recognition of the existence of plasma membrane frequency-dependent capacitances is critical in the design and interpretation of studies of epithelial tissues. It is of fundamental interest to know the factors and mechanisms underlying audio frequency dielectric dispersions or relaxation processes in epithelial and nonepithelial plasma membranes in terms of the organization of the lipids and proteins of plasma membranes and their structural/functional relationships that give rise to dielectric dispersions at any frequency, and in particular those at audio frequencies (\(\alpha\) dispersions (Schwan, 1957)) where transepithelial impedance is usually measured. To the extent that plasma membranes exhibit Cole-Cole-like dielectric dispersions (Cole and Cole, 1941) and to the extent additionally that apical and basolateral membranes form a series arrangement of leaky dielectrics leading to Maxwell-Wagner dispersions, interpretation of changes of capacitance is more complex, requiring consideration not
only of membrane areas and thicknesses but also those factors that can change the frequency-dependent dielectric properties of the membrane and hence, the capacitance measured at any frequency.

THEORETICAL CONSIDERATIONS

Electrical equivalent circuits

In the face of nonlinear current-voltage relationships of the underlying channels and electrodiffusive transporters, impedance analysis requires electrical equivalent circuits where membranes are modeled by their slope resistances \( R \) and capacitances \( C \) (as compared to \( g \)'s in the chord formalism), and used \( E \)'s for Thévenin emfs that are not necessarily the same as the \( C \)'s that designate Nernst equilibrium potential differences. Thus, as indicated in Fig. 1 A, the epithelium is modeled as a series arrangement of apical and basolateral membrane impedances that exists in parallel with a paracellular shunt resistance, \( R_p \). The respective slope resistances \( R_a \) and \( R_b \) and capacitances \( C_a \) and \( C_b \) of apical and basolateral membranes give rise to the apical and basolateral membrane impedances \( Z_a \) and \( Z_b \), so that the transepithelial impedance \( Z_T \) is:

\[
Z_T = \frac{(Z_a + Z_b)R_p}{Z_a + Z_b + R_p}
\]  

(1)

FIGURE 1  (A) Transepithelial lumped parameter electrical equivalent circuit of apical and basolateral plasma membranes of the cells shunted by a paracellular resistance, \( R_p \), \( a \), and \( c \) indicate nodes within the apical, basolateral, and intracellular solutions, respectively. \( E_a \) and \( E_b \) are Thévenin emfs of apical and basolateral membranes, respectively, with their associated slope resistances, \( R_a \) and \( R_b \), and respective capacitances, \( C_a \) and \( C_b \).Because impedance is measured in the time domain, the conductances must be slope conductances at the operating point voltages of the membranes. Shown are Goldman \( V - I \) relationships (Goldman, 1943) for apical membrane Na\(^+\) and Cl\(^-\) channels at the operating point \( (V_a^e) \) of short-circuited epithelia that pertain to the conditions of our experiments. Nernst equilibrium potential differences for Na\(^+\) \( (E_{Na}) \) and Cl\(^-\) \( (E_{Cl}) \) are indicated at the points of current reversal. The drawing indicates that whereas Cl\(^-\) is at or very near electrochemical equilibrium, Na\(^+\) transport is finite at an apical membrane voltage \( (V_a^e) \) far removed from equilibrium in short-circuited tissues \( (V_a^c) \) to emphasize that the operating point voltage for Cl\(^-\) is close to electrochemical equilibrium, whereas the distribution for Na\(^+\) across the apical membrane is far removed from its electrochemical equilibrium with a Thévenin emf, \( E_{Na} \), at or very near zero. From the point of view of impedance, the emfs (Nernst or Thévenin) are irrelevant, but the slope conductances will vary with voltage due to the degree of nonlinearity of the \( V - I \) relationships at the operating points of membrane voltage.
The notation of asterisk-superscripted C’s indicates that capacitance may be complex (frequency-dependent). If the C’s are complex, then the time constants, $RC^*$, are also frequency-dependent. At the limiting frequency bounds, $Z_T$ approaches zero at infinite frequency; as frequency approaches zero, $Z_T$ approaches the transepithelial slope resistance, $R_T = [(R_a + R_b)R_p]/(R_a + R_b + R_p)$. If the capacitances at the audio frequencies of impedance measurements exhibit Maxwell-Wagner and/or Cole-Cole dispersions (Awayda et al., 1999) (or dielectric dispersions from any other source), such dispersions will give rise to power law dependence of the impedance (see below).

Shown also in Fig. 1 are the electrodiffusive or conductive components of $R_b$ in three transport states of the tissues. In their control state (Fig. 1 C), A6 epithelia, otherwise untreated by hormones or second messengers that activate Cl⁻ channels, express both blocker-sensitive ($b$) and to a much lesser extent blocker-insensitive ($bi$) Na⁺ currents that account completely for the total current or the net rates of Na⁺ entry into the cells as measured by short-circuit currents (Baxendale-Cox et al., 1997; Helman et al., 1998). As indicated, $R_b$ is partitioned between blocker-sensitive ($R_{Na}^{bi}$) and blocker-insensitive ($R_{Na}^{bi}$) slope resistances. Notably, the Thévenin emfs of these Na⁺ conductances ($E_{Na}^{bi}$) are at or very near zero, because as indicated in Fig. 1 B, the slope conductance, $G_{Na}$ at the operating point of the apical membrane voltage ($V_{a}^{bi}$) intersects the voltage axis at or very near zero when $V_{a}^{bi}$ is very far removed from electrochemical equilibrium ($E_{Na}$) (Helman and Thompson, 1982). Accordingly, the blocker-sensitive and -insensitive Na⁺ currents are $I_{Na}^{bi} = V_{a}^{bi}R_{Na}^{bi}$ and $I_{Na}^{bi} = V_{a}^{bi}R_{Na}^{bi}$. With amiloride block of apical membrane ENaCs, the apical membrane $R_a$ reduces to the $R_{Na}^{bi}$, as indicated in Fig. 1 D. With $R_{Na}^{bi}$ averaging much less than 0.5 $\mu$A/cm² (Helman and Liu, 1997; Baxendale-Cox et al., 1997; Blazer-Yost et al., 1999; Păunescu et al., 2000) and $V_{a}$ exceeding 100 mV under these conditions, the $R_{Na}^{bi}$ is expected to be far greater than 200,000 $\Omega \cdot$ cm².

Activation of apical membrane Cl⁻ channels introduces a slope conductance $G_{Cl} = 1/R_{Cl}$ with a Thévenin $E_{Cl}$. If, as indicated in Fig. 1, $B$ and $E$, $Cl$ is distributed across the apical membrane at or very near the Nernst equilibrium potential difference, $E_{Cl}$, then $E_{Cl}$ can be replaced by $E_{Cl}$. To the extent that $R_{Na}^{bi} \gg R_{Cl}$, $R_a \approx R_{Na}$ at the operating point of the apical membrane voltage, which as indicated elsewhere (Păunescu and Helman, 2001) is very close to $E_{Cl}$.

For purpose of discussion below we have used values of resistance and capacitance in the range(s) that we believe are relevant to those of A6 epithelia studied in our laboratory (Liu and Helman, 1998; Păunescu et al., 2000; Păunescu and Helman, 2001). In amiloride-blocked states of Na⁺ transport, $R_a = R_{Na}^{bi}$ is near 500 k$\Omega \cdot$ cm² and $R_b$ averages near 4000 $\Omega \cdot$ cm². $R_b$ may vary with the rate of Na⁺ transport (Păunescu et al., 2000) but there is presently insufficient information to know if this is so in A6 epithelia and to what extent. The $R_b$ of control tissues is highly variable and depends on the rate of Na⁺ transport (short-circuit currents) where the spontaneous amiloride-sensitive $I_{sc}$ can range downward to values <1 $\mu$A/cm² ($R_{Na}^{bs} > 112$ k$\Omega \cdot$ cm²) and upward to >30 $\mu$A/cm² ($R_{Na}^{bs} < 3.7$ k$\Omega \cdot$ cm²). The static dc or zero-frequency capacitances of apical ($C_{a}^{bi}$) and basolateral membranes ($C_{b}^{bi}$) average near 1.5 and 20 $\mu$F/cm², respectively (Păunescu and Helman, 2001). Thus, for the monolayers of A6 epithelia where basolateral membrane area is considerably less than in the functionally coupled cells of the multicellular layers of frog skin, the basolateral membrane resistance of A6 epithelia is about four times greater and the capacitance about three times less than observed in studies of frog skin (Awayda et al., 1999). Accordingly, and unlike in frog skin, where the impedance of the basolateral membranes under some experimental conditions may approach negligible values, the $Z_b$ of A6 epithelia cannot be neglected at any frequency, as will be indicated below.

Interpretation of transepithelial impedance data and the design of experiments to evaluate the tissues is complicated not only by the juxtaposed impedance of the cells ($Z_a + Z_b$) and their paracellular shunts ($R_p$), but also by the possibility that the plasma membranes exhibit capacitive dispersions at the frequencies of interest. A simplification of experimental conditions utilizes amiloride to block apical membrane epithelial Na⁺ channels (ENaCs) so that at least at very low frequencies, $Z_a \gg Z_b$ and the cellular resistance ($R_{cell} = R_a + R_b$) is much greater than $R_p$. Accordingly, with $R_a \gg R_b$ and $R_{cell} \gg R_p$, the measured transepithelial resistance, $R_T$, closely approaches the values of $R_p$. At very high frequencies, where the $R_a$ and $R_b$ become negligible relative to their respective capacitive reactances, the equivalent capacitance of the cells, $C_{eq}^{b}$, approaches values of the series arrangement of capacitance ($C_{a}^{bi} + C_{b}^{bi})$, with $C_{eq}^{b}$, which notably such values would not be the same as ($C_{a}^{bi} + C_{b}^{bi})/(C_{a}^{bi} + C_{b}^{bi})$ if either or both apical and basolateral membranes exhibit dielectric dispersions. Thus, we have considered in more detail the behavior of the transepithelial impedance in amiloride-treated tissues first. Also, it will be evident below that it is critically important to know with reasonable certainty the values of $R_p$ especially under conditions where $R_a$ is not much greater than $R_b$ and $R_p$, as would be the case for tissues transporting Na⁺ or for tissues in their amiloride or non-amiloride pretreated state where cAMP activates apical membrane Cl⁻ channels.
FREQUENCY-DEPENDENT CAPACITANCE

Maxwell-Wagner dispersions

Although the measurement of impedance is straightforward, the analysis and interpretation of data is complicated when capacitances are frequency-dependent. Frequency-dependent capacitances may arise from Maxwell-Wagner dispersions (Daniel, 1967; Jonscher, 1983) due to the juxtaposition of leaky dielectrics that exist in series. From the transepithelial point of view, the apical and basolateral membranes of the cells behave as a series arrangement of leaky dielectrics with capacitances $C_a$ and $C_b$ paralleled by resistances $R_a$ and $R_b$, respectively, as indicated in Fig. 1 A. Accordingly, the transcellular impedance behaves as a complex frequency-dependent equivalent capacitance, $C_{eq}^*$, where:

$$C_{eq}^* = \frac{1}{j\omega(Z_a + Z_b)}$$  \hspace{1cm} (4)

Nyquist and Bode plots of $C_{eq}^*$ are plotted in Fig. 2. For the case where $R_a$ is infinite and where $C_a^{dc} = 1.5 \text{ }\mu\text{F/cm}^2$, $C_b^{dc} = 20 \text{ }\mu\text{F/cm}^2$, and $R_b = 4000 \text{ }\Omega\cdot\text{cm}^2$, $C_{eq}^*$ decreases from the $C_a^{dc}$ of 1.5 $\mu$F/cm$^2$ to 1.395 $\mu$F/cm$^2$ as frequency increases toward $\sim 100–200$ Hz. Despite the fact that $C_a$ and $C_b$ are constant at all frequencies, the series arrangement of membranes gives rise to a Maxwell-Wagner dispersion at low audio frequencies so that the cells behave as a frequency-dependent capacitance that exists in parallel with the $R_p$. The dispersion appears as an ideal semicircle in Nyquist plots.

FIGURE 2 Nyquist (A) and Bode plots (B and C) are shown for the equivalent complex capacitance, $C_{eq}^*$, of a transcellular Maxwell-Wagner dispersion if $R_a = \infty$. Maxwell-Wagner plots of $C_{eq}^*$ are shown in (D) (Nyquist) and (E) and (F) (Bode) when $R_a = 100 \text{ }\Omega\cdot\text{cm}^2$ and 500 $\text{k}\Omega\cdot\text{cm}^2$, $R_b = 4000 \text{ }\Omega\cdot\text{cm}^2$, $C_a = 1.5 \text{ }\mu\text{F/cm}^2$, $C_b = 20.0 \text{ }\mu\text{F/cm}^2$. The capacitances are assumed to be independent of frequency.
plots with a characteristic frequency at the apex of the semicircle of 1.79 Hz, as indicated in Fig. 2 A. When $R_a$ is not infinite and in the range of 100–500 kΩ · cm$^2$ as illustrated in Fig. 2 D, the $C_{eq}$ due to Maxwell-Wagner dispersions are quantitatively more complex, reflecting the fact that a finite value of resistance behaves in the equivalent sense as a frequency-dependent capacitance ($C(\omega) = (j\omega R)^{-1}$). Accordingly, as illustrated in Fig. 2, D–F, the imaginary components of $C^*(ImC_{eq})$ become enormous in value relative to those of the real components of $C_{eq}$ ($ReC_{eq}$) as frequency decreases toward zero. Thus, despite relatively high values of $R_a$ in amiloride-blocked tissues, such values of $R_a$ will markedly affect the magnitudes and phases of the $C^*$ at low frequencies < $\sim$100 Hz for the parameters of the circuit used here in our calculations (Fig. 2, E and F). If capacitances do not exhibit dielectric dispersions at higher frequencies than $\sim$100 Hz, then with $C_a = 1.5$ μF/cm$^2$ and $C_b = 20$ μF/cm$^2$, $C_{eq}$ would be 1.395 μF/cm$^2$ at higher frequencies beyond those expected of the Maxwell-Wagner dispersions. Indeed, because $C_a$ is considerably larger than $C_b$, the Maxwell-Wagner frequency-dependent decrement of $C_{eq}^*$ is relatively small (7%), but nevertheless important at the lower audio frequencies where attempts are made to determine the $dc$ capacitances. Such considerations are especially important in those tissues that spontaneously transport Na$^+$ at low rates, in tissues where Na$^+$ transport is suppressed by drugs, hormones, or other experimental maneuvers that affect $R_{eq}^{Na}$ and in particular in those tissues treated with amiloride to completely inhibit blocker-sensitive apical membrane Na$^+$ channels. With shunt resistances in the range of 10 kΩ · cm$^2$, the useful frequency range of interest (see below) includes those frequencies down to $\sim$0.1 Hz, where Maxwell-Wagner dispersions will affect the magnitudes and phases of the transepithelial impedance. It must be stressed, owing to the series arrangement of apical and basolateral membranes of epithelia in general, that Maxwell-Wagner dispersions are unavoidable regardless of the magnitudes of conductances and capacitances in tight and leaky epithelia and regardless of additional dispersions that may arise from the plasma membrane dielectrics.

Thus, independent of the values of $R_a$, interpretation of measurements of impedance can be problematic at very low frequencies due to unavoidable Maxwell-Wagner dispersions.

### Cole-Cole dielectric dispersions

In contrast to Maxwell-Wagner dispersions, frequency-dependent capacitances can also arise from dielectric relaxation processes as described originally by Cole and Cole, Schwan, and others (Cole and Cole, 1941; Schwan, 1957; Kell and Harris, 1985; Takashima, 1989) and can be associated with $C_a$ and/or $C_b$. When observed at audio frequencies these relaxation processes are referred to as α dispersions, while those at higher radio frequencies have been referred to as β dispersions. Our considerations in this paper will be limited to α dispersions. Plasma membranes may exhibit multiple relaxation processes (Eq. 5) (Cole and Cole, 1941; Awayda et al., 1999) where the complex capacitance $C^*$ at any frequency will vary with the magnitudes of the dielectric increments ($C_i$), the time constants ($\tau_i$), or characteristic frequencies ($f_i$) of the relaxation processes and power law coefficients ($\alpha_i$) that lead to observation of depressed semicircles in Nyquist plots of $C^*$. $C_{eq}$ represents the limiting static capacitance at frequencies far greater than the highest relaxation frequency process ($f_1 = (2\pi \tau_1)^{-1}$) that exists in the audio range of frequencies.

Resolving plasma membrane dielectric relaxation processes in intact epithelial tissues at high frequencies is limited by the uncertainties in measurement of the exact magnitude of the series solution resistance of the cell cytoplasm and the solutions bathing the apical and basolateral borders of the tissues. With high values of capacitance (1.5 μF/cm$^2$), the capacitive reactance of $C_a$ at 10 kHz is 10.6 Ω · cm$^2$ and only 1.06 Ω · cm$^2$ at 100 kHz, which approaches the value of the cytoplasmic resistance of the cells. Thus, detection of dispersions that exist in the range of radio frequencies (β dispersions) or higher frequencies is essentially if not completely precluded because the errors in determining $Z_1$ from small differences between the measured impedances and those of the series resistances of the cells and bathing solutions cannot be adequately assessed. Thus, our experiments and our considerations are limited to audio frequency α dispersions of the type described by Eq. 5.

$$C^* = \sum_{i=1}^{n} \frac{C_i}{1 + (j\omega \tau_i)^\alpha} + C_{eq}^a$$  (5)

Illustrated in Fig. 3, A–C are Nyquist and Bode plots of a single relaxation process. We have assumed, based upon our experimental findings (Pâunescu and Helman, 2001) that the apical membrane is characterized by a Cole-Cole relaxation process, so that the complex apical membrane capacitance, $C_{eq}^a$, can be calculated with Eq. 5. The $dc$ capacitance, $C_{eq}^{dc}$, is 1.5 μF/cm$^2$ (Pâunescu and Helman, 2001). The dielectric increment, $C_{1a} = C_{eq}^{dc} - C_{eq}^{a}$, is 0.6 μF/cm$^2$ with a relaxation frequency of 110 Hz. The complex capacitance approaches $C_{eq}^a = 0.9$ μF/cm$^2$ as frequency far exceeds 10 kHz approaching values near 1 MHz. The semicircle is depressed due to a power law dependence of the capacitance, $\alpha_a = 0.6$, which is consistent with the idea that there is a distribution of time constants (Cole and Cole, 1941; Cole, 1968; Gabler, 1978; Pethig, 1979; Jonscher, 1983) of the relaxation processes centered at a relaxation frequency of 110 Hz. For such dispersions the complex capacitance vectors vary in magnitude ($|C_{eq}^a|$) and phase angle (φ) as indicated in the Nyquist plots (Fig. 3 A) or in rectangular
coordinates as the real (\(\text{Re}C_a\)) and imaginary (\(\text{Im}C_a\)) components of \(C_{eq}\) as indicated in Fig. 3, B and C, respectively. The absolute magnitude of \(\text{Im}C_a\) is maximal at the relaxation frequency of 110 Hz (Fig. 3 C). The \(\text{Re}C_a\) is decreased from \(C_\infty\) by one-half of the magnitude of the dielectric increment (\(C_{1\alpha} = C_\infty - C_a\)) at 110 Hz (Fig. 3 B). It should be noted that \(\alpha_a = -2\text{Im}C_a/C_\infty \) where \(0 \leq \alpha_a \leq 1\).

**Combined Maxwell-Wagner and Cole-Cole dispersions**

With Maxwell-Wagner dispersions dominant at lower frequencies and \(\alpha\) dispersions at higher frequencies, resolving the \(\alpha\) dispersions at lower frequencies is complicated, as illustrated in Fig. 3, D–F. Regardless of the values of \(R_a\) and assuming for the sake of simplicity that \(C_b\) is devoid of \(\alpha\) dispersions, the behavior of \(C_{eq}\) at frequencies \(<\sim100–200\) Hz is markedly influenced by the Maxwell-Wagner dispersion. If, additionally, \(C_b\) exhibits \(\alpha\) dispersions, the behavior of \(C_{eq}\) would be more complex especially at the lower frequencies. Consequently, from the transepithelial point of view, capacitance of the cells would appear frequency-dependent over the entire range of audio frequencies, where at higher frequencies the changes of capacitance would be dominated by the \(\alpha\) dispersions but with more complex behavior at the lower frequencies due to the Maxwell-Wagner dispersion. Assuming, as indicated above, that \(C_b\) (20 \(\mu F/cm^2\)) is devoid of \(\alpha\) dispersions, the \(C_{eq}\) is 0.861 \(\mu F/cm^2\) (Fig. 3, D and E) and the \(C_{eq}\) would be 1.395 \(\mu F/cm^2\) if \(R_a\) and \(R_b\) were infinitely large, as indicated by the dashed lines in these figures. The contribution of the Maxwell-Wagner dispersion to \(C_{eq}\) can be assessed by examination of the differences of \(C_{eq}\) when \(R_a\) and \(R_b\) are either finite or infinite in value.
**Determination of the complex equivalent capacitance, \( C_{eq}^* \)**

\( C_{eq}^* \) is best determined under conditions where ideally \( R_a \) is infinitely high so that the transepithelial resistance is due solely to the shunt resistance, \( R_p \). Under such conditions the transepithelial impedance is determined by the parallel combination of \( R_p \) and \( C_{eq}^* \) so that:

\[
Z_T = \frac{R_p}{1 + j\omega R_p C_{eq}^*}
\]  

(6)

Rearranging Eq. 6 allows calculation of the \( C_{eq}^* \) from the measured values of \( Z_T \). [It should be noted according to Eq. 6 and the definition of the complex capacitance, \( C_{eq}^* \) in Eq. 4 that the transepithelial impedance of an epithelium can always be modeled by an \( R_p \) paralleled by the cellular impedance \((Z_a + Z_b)\) regardless of the tightness or leakiness of the junctional complexes in the paracellular shunt pathways of the epithelium. To the extent that \( R_a \) is practically infinitely large, as in amiloride-blocked tissues with unstimulated intracellular levels of cAMP, \( R_p \) can be equated directly with the \( R_T \) and determined from the measured values of \( Z_T \) as frequency approaches zero, thereby simplifying analysis, as \( R_p \) can be measured directly. When \( R_a \) cannot be neglected, \( R_p \) must be determined by an independent method because \( R_p > R_T \). To the extent that \( R_a \) can vary over wide ranges depending on the rates of \( Na^+ \) transport, it is always possible to use amiloride to block \( Na^+ \) channels \((R_{Na}^{bs} \to \infty)\) thereby elevating \( R_p \) to values of \( R_{Na}^{bi} \), that will exceed 200 k\( \Omega \cdot \)cm\(^2\) (see above) when the amiloride-insensitive \( Na^+ \) currents are <0.5 \( \mu A/cm^2\). Under these amiloride-blocked conditions where \( R_a \gg R_p \), the transepithelial resistance, \( R_T \), approaches values of the parallel combination of \( R_a \) and \( R_p \) or values of \( R_p \) when \( R_a \gg R_p \). Thus, with \( R_p \) averaging near 1 \( k\Omega \cdot \)cm\(^2\) and cell resistances much greater than 200 \( k\Omega \cdot \)cm\(^2\), \( C_{eq}^* \) determined with Eq. 6 would to a very good approximation provide data that would allow discrimination between dispersions arising from Maxwell-Wagner behavior and dielectric relaxation processes. Plotted in Fig. 4 are the magnitudes, \( |C_{eq}^*| \), and phase angles, \( \phi \), of \( C_{eq}^* \) for \( R_p \) between 100 \( k\Omega \cdot \)cm\(^2\) and infinity attributable alone to the Maxwell-Wagner dispersion. At frequencies \( \gtrsim 10 \) Hz, \( |C_{eq}^*| \) is constant. However, at frequencies \( <10 \) Hz, \( |C_{eq}^*| \) increases precipitously as frequency decreases at finite values of \( R_a \). Accordingly, despite block of the amiloride-sensitive \( Na^+ \) channels, the Maxwell-Wagner effect makes it appear as though capacitance exceeds the actual \( C_{eq}^* \) of 1.5 \( \mu F/cm^2\). This is a pitfall of the analysis where what seemingly may be a reasonable assumption, namely a very high and “negligible” value of \( R_a \) relative to \( R_p \) and \( C_{eq}^* \) is in fact a poor assumption when analysis of data is carried out at low frequencies (see below) in the range of the Maxwell-Wagner dispersions. Not only are the \( |C_{eq}^*| \) overestimated at frequencies \( <10 \) Hz, the phase angles of \( C_{eq}^* \) also vary with frequency up to 1 MHz when \( R_a \) is 100 \( k\Omega \cdot \)cm\(^2\) (Fig. 4 B). It is important to realize that with \( R_p \)'s in the range of 10 \( k\Omega \cdot \)cm\(^2\) the characteristic frequencies of impedance \( f^2 = (2\pi R_p |C_{eq}^*|)^{-1} \) are in the range of 10 Hz, so that impedance measured at these lower frequencies is especially sensitive to changes of capacitance associated with Maxwell-Wagner dispersions.

Although the Maxwell-Wagner effect on \( |C_{eq}^*| \) is dominant at low frequencies, the dielectric dispersions are readily distinguished from those of Maxwell-Wagner at higher frequencies, as indicated in Fig. 5 A. For the relaxation process centered at 110 Hz, which we have used in our considerations of A6 epithelia, \( |C_{eq}^*| \) are virtually the same at frequencies \( \gtrsim 30–40 \) Hz irrespective of the values of \( R_a \) 100 \( k\Omega \cdot \)cm\(^2\). An analysis such as this can and should...
be done for any set of parameters. Our calculations presented here apply only to those values that we have reason to believe are representative of A6 epithelia studied in our own laboratory.

We emphasize that our analysis for the purpose of discussion presumes that an \( \alpha \) dielectric dispersion occurs only at the apical membranes of the cell. Quite generally, there may be more than one \( \alpha \) dispersion at apical membranes and it is likely that basolateral membranes also exhibit \( \alpha \) dispersions. Presently, we have no specific information on the dielectric behavior of any basolateral membrane. It is nevertheless clear that with values of \( C_b^{dc} \) much greater than those of \( C_a^{dc} \) (20 vs. 1.5 \( \mu F/cm^2 \)), the contribution of basolateral membrane dielectric dispersions to the values of \( C_{eq} \) would be relatively small compared to those arising from the apical membrane if the dispersions at both membranes are proportionally the same. Thus, it is more likely that if \( \alpha \) dispersions are observed under the conditions considered here, they arise from the apical membranes of the cells (however, see also below).

It should be recognized that A6 epithelia are capable of wide ranging rates of spontaneous Na\(^{+}\) transport with lower limits near 1 \( \mu A/cm^2 \). In such tissues the values of \( R_a \) will be near 100 k\( \Omega \cdot cm^2 \). Consequently, from the point of view of transepithelial impedance, such low transport rate tissues will behave more like amiloride-blocked tissues. However, when apical membrane conductance to Na\(^{+}\) is spontaneously high or when Cl\(^-\) conductance is activated by cAMP in the presence or absence of amiloride, it would no longer be possible to assume, as above, that values of \( R_a \) approach values of \( R_{eq} \). \( R_a \) may also be either larger or smaller than \( R_{eq} \). Such conditions impose special limitations in evaluation of the frequency-dependent characteristics of the plasma membranes (see below). Consequently, our discussion to follow focuses on experimental conditions where \( R_a \gg R_{eq} \), and in particular on the influence of the \( R_p \) on the analysis of experimental data.

**IMPEDEANCE**

**Depressed impedance loci (semicircles) in Nyquist plots \( (R_a \gg R_b) \)**

It has been a consistent and inviolate observation in our laboratory (Liu and Helman, 1998; Awayda et al., 1999; Păunescu and Helman, 2001) and others (Schifferdecker and Fromter, 1978; Gordon et al., 1989) that Nyquist impedance plots invariably exhibit depressed semicircular behavior regardless of the state of transport of the epithelium studied \( (R_a > R_{eq}; R_a < R_b) \), requiring fitting equations with power law dependence to account for the depressed locus of the impedance vectors. Complex frequency-dependent capacitances give rise to power law dependencies of impedance as indicated below. Eq. 6 can be rewritten as:

\[
Z_T = \frac{R_p}{1 + j\omega R_p(ReC_{eq} - j\cdot ImC_{eq})} \tag{7}
\]

which can be expanded in rectangular coordinates \((ReZ_T - j\cdot ImZ_T)\) to:

\[
Z_T = \frac{1 + (\omega R_p ImC_{eq}^{*})}{(1 + \omega R_p ImC_{eq}^{*})^2 + (\omega R_p ReC_{eq}^{*})^2} - j\frac{\omega R_p ReC_{eq}^{*}}{(1 + \omega R_p ImC_{eq}^{*})^2 + (\omega R_p ReC_{eq}^{*})^2} \tag{8}
\]
At the frequency $\omega = 2\pi f^2 = (R_p|C_{eq}^\ast|)^{-1}$:

$$f^2 = \frac{1}{2\pi R_p \sqrt{\text{Re}C_{eq}^\ast + \text{Im}C_{eq}^\ast}}$$  \hspace{1cm} (9)

and it is readily shown that the $\text{Re}Z_T = R_p/2$ when $f = f^*$, regardless of the value of the $\text{Im}Z_T$ (or regardless of the degree of depression of the impedance locus). Defining the degree of depression of the Nyquist impedance semicircle, $\gamma$, as $\text{Im}Z_T/\text{Re}Z_T = 2 \cdot \text{Im}Z_T/R_p$ at $f = f^*$, $\gamma$ is:

$$\gamma = \frac{\text{Re}C_{eq}^\ast}{\sqrt{\text{Re}C_{eq}^\ast + \text{Im}C_{eq}^\ast + \text{Im}C_{eq}^\ast} |_{f = f^*}}$$  \hspace{1cm} (10)

Consequently, depression of the impedance semicircles in Nyquist plots is independent of the magnitude of $R_p$. It should be noted and emphasized that depression of the impedance locus requires $|\text{Im}C_{eq}^\ast| > 0$, like those that arise with Maxwell-Wagner and Cole-Cole $\alpha$ dispersions. It should be appreciated that if $\text{Im}C_{eq}^\ast$ is zero, then change alone of $\text{Re}C_{eq}^\ast$ does not give rise to a depressed impedance locus.

To illustrate the relative importance of the $\text{Im}C_{eq}^\ast$ and $\text{Re}C_{eq}^\ast$ on depression of the impedance locus we calculated $\gamma$ for a simple parallel combination of an $R_p$ and an apical membrane with a single Cole-Cole relaxation process where $C_a^\infty$ was 1.5 $\mu$F/cm² and where $C_{eq}^\ast$ was 0.5, 0.9, or 1.2 $\mu$F/cm², thereby giving dielectric increments of 1.0, 0.6, and 0.3 $\mu$F/cm², respectively, at characteristic frequencies of 110 Hz, as above. Depression of capacitance, $\alpha_a$, was varied between extremes of 0 and 1.

The relationship between $\alpha_a$ and $\gamma$ is nonlinear and inverse as illustrated in Fig. 6. The degree of depression of the impedance locus will vary not only with the magnitude of the dielectric increment but also requires, as indicated above, that $\alpha_a > 0$ (or $\text{Im}C_{eq}^\ast \neq 0$). It is also apparent that the maximal influence of the capacitive dispersion occurs when $\alpha_a$ approaches unity. Although we have dealt here with specific values to illustrate a point, this discussion can be generalized to any frequency-dependent dielectric behavior (and to Maxwell-Wagner behavior) which, according to the generalized thesis presented above (Eqs. 7–10), may be used to argue for the existence of frequency-dependent capacitances when the impedance locus is depressed.

Depressed Nyquist impedance loci are plotted in Fig. 7-A for epithelia with apical and basolateral membrane characteristics described above. The $\alpha_a$ are 0.25, 0.5, 0.75, and 1.0 and $R_p$ is 1000 $\Omega \cdot \text{cm}^2$ ($C_{eq}^d = 1.5 \mu$F/cm²; $C_{eq}^\infty = 0.9$ $\mu$F/cm²; $f^* = 110$ Hz; $R_a = 500$ k$\Omega \cdot \text{cm}^2$; $R_b = 4000$ $\Omega \cdot \text{cm}^2$; $C_b = 20$ $\mu$F/cm²). Depression of $\text{Im}Z_T$ is maximal at $\alpha_a = 1.0$ (Fig. 7, A and C) at frequencies near 142 Hz for $\alpha_a = 0.25$, 0.50, and 0.75 and near 148 Hz for $\alpha_a = 1.0$ (Fig. 7 C). The corresponding values of $\gamma$ and $\alpha_a$ are given in Fig. 7 C; $\gamma$ ranges between 0.785 and 0.953 for $\alpha_a$ between 1.0 and 0.25. Although the maximal absolute values of $\text{Im}Z_T$ exist at frequencies very close to those expected when $\text{Re}Z_T = R_p/2$, the small deviations from ideal depressed semicircles exist because of the greater contribution of the Maxwell-Wagner dispersion to the impedance at the lower frequencies leading to an asymmetry in the impedance locus.

The exact impedance locus will depend not only on the capacitive dispersions but also on the values of $R_p$, because $R_p$ is a major component of transepithelial impedance that will determine the frequencies at $\text{Re}Z_T = R_p/2$. For $R_p$’s of 1, 10, and 50 k$\Omega \cdot \text{cm}^2$ (Fig. 8, A–C), the corresponding frequencies are 142.2, 12.09, and 2.45 Hz. Thus, in tissues with very high $R_p = 50$ k$\Omega \cdot \text{cm}^2$ (Fig. 8 B), the principal changes of $Z_T$ occur over a very small bandwidth (0.1 to 10 Hz) that is primarily in the frequency range of the Maxwell-Wagner dispersion. When $R_p = 1$ k$\Omega \cdot \text{cm}^2$ (Fig. 8 A), the major changes of impedance occur over a range of frequencies (10 Hz–1 kHz) in the range of the $\alpha$ dispersion. At an intermediate $R_p = 10$ k$\Omega \cdot \text{cm}^2$ (Fig. 8 C), the major changes of $Z_T$ occur between 0.1 Hz and ~50 Hz, thereby encompassing the Maxwell-Wagner dispersion and a relatively small portion of the $\alpha$ dispersion. Thus, it should not be surprising, especially at these intermediate values of $R_p$, that the impedance loci cannot be described exactly by a simple equation of a single depressed semicircle.

For experimental data that appear to conform by inspection to an ideal (non-skewed) depressed semicircle, it is tempting to fit the data to a general and empirical equation of the form (Van Driessche, 1986):

$$Z_T = \frac{R_0^{\text{fit}}}{1 + (\omega/R_0^{\text{fit}} C_{eq}^{\text{fit}})^{\gamma^2}}$$  \hspace{1cm} (11)

Such an equation assumes that the impedance locus is symmetrical at frequencies above and below the frequency at the apex of the semicircle. The capacitance, $C_{eq}^{\text{fit}}$, is the capacitance at $\omega = (R_0^{\text{fit}} C_{eq}^{\text{fit}})^{-1}$. However, depending on the degree of asymmetry of the semicircle, the values of $R_0^{\text{fit}}$ may be larger or smaller than those of $R_T$, depending on the
range of $Z_T$ included in fitting data to Eq. 11. Thus, for example, if the $Z_T$ between 0.1 Hz and 50 Hz in Fig. 8 C are fit to Eq. 11, the fitted dashed line deviates from the actual data at frequencies $>50$ Hz (Fig. 8 D) and the value of $C_{eq}^{fit}$ approximates the value of $|C_{eq}^*>$ at 12.09 Hz. Such fitting provides no information on the Maxwell-Wagner or $\alpha$ dispersions. Capacitance is calculated at a singular value of frequency at the apex of the fitted semicircle, where the frequency is determined not only by the capacitance, but also in particular by the value of $R_p$. Consequently, if $R_p$ changes, then capacitance will appear to change due to the change of $R_p$ and not due to change of the dielectric properties or area changes of the plasma membranes. For the conditions illustrated in Fig. 8 ($R_p = 1, 10, and 50 \, k\Omega \cdot cm^2$), the values of $C_{eq}^{fit}$ are remarkably close to the actual values of $|C_{eq}^*>$ at the frequencies, $f^*$.

It is also important to know that in the face of frequency-dependent capacitances there are experimental circumstances where impedance loci cannot be approximated by Eq. 11. For example, as illustrated in Fig. 9, we have calculated the impedance at values of $R_p$ of 200, 600, and 1000 $\, \Omega \cdot cm^2$ assuming, ideally, that $R_a = \infty$ and that $\alpha_a = 1.0$. Inspection of the plots in Fig. 9 A indicates clearly that it would be inappropriate to attempt to fit such data to Eq. 11, as the behavior of the impedance loci is certainly more complicated. This behavior, although influenced by the basolateral membrane $R_b$ and $C_b$, is not due to a Maxwell-Wagner effect but rather to the apical membrane relaxation process as illustrated in Fig. 9 B, where the impedance loci were calculated with $R_b = 0$. Accordingly, the exaggerated asymmetry of the impedance loci in Fig. 9 B is due alone to the apical membrane dispersion that would be observed prevalently in “leaky” epithelia. If fitting of data at the higher frequencies is attempted with Eq. 11, the capacitances calculated would reflect those of the $\alpha$ dispersions at frequencies near 963 Hz when $R_p = 200 \, \Omega \cdot cm^2$.

If it is assumed that capacitance is constant at all frequencies, then it would be tempting to believe that data like those in Fig. 9 are best fit with a mathematical model consisting of an apical membrane $R_aC_a$ and basolateral membrane $R_bC_b$ that exist in parallel with $R_p$ (see below). However, this would clearly be inappropriate here and would represent a serious pitfall when plasma membranes exhibit $\alpha$ dispersions. Thus, to avoid this pitfall, it would be prudent to evaluate epithelia for the existence of dispersions by any method appropriate to their transport physiology. For A6 epithelia and other tight epithelia where apical membranes express only Na$^+$ channels, the amiloride-blocked state of the tissues serves as such a method. Similarly, for very leaky tissues where $R_p \ll (R_a + R_b)$, frequency dependence due to Maxwell-Wagner or $\alpha$ dispersions can be assessed when the conditions of the experiments conform to Eq. 6.

**Activation of apical membrane conductance**

In response to second messengers like cAMP, the apical membrane conductances to Na$^+$ and Cl$^-$ are activated in tissues like A6 epithelia resulting in decrease of $R_a$ and hence decrease of the fractional transcellular resistance $R_{eq} = R_a/(R_a + R_b)$. Depending on the relative values of $R_a$ and $R_b$ and their associated values of $C_a^*$ and $C_b^*$, the transcellular impedance loci will, according to their time constants $R_aC_a^*$ and $R_bC_b^*$, reflect the existence of two plasma membranes in series by partially overlapping semicircles in Nyquist impedance plots dictated by the magnitudes of the impedance vectors of apical and basolateral membranes and the $R_p$ of the paracellular shunt. Under absolutely ideal conditions when $R_p = \infty$ and $C_a$ and $C_b$ are constant at all frequencies, the transcellular impedance locus would appear as illustrated in

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**FIGURE 7** Influence of an apical membrane Cole-Cole dispersion on the transepithelial impedance ($Z_T$). $R_p = 1000 \, \Omega \cdot cm^2$, $R_a = 4000 \, \Omega \cdot cm^2$, $C_a = 20.0 \, \mu F/cm^2$, $C_a^{eq} = 1.5 \, \mu F/cm^2$, $C_b = 0.9 \, \mu F/cm^2$, $f^* = 110 \, Hz$. Calculations were done with $\alpha_a = 0.25, 0.5, 0.75,$ and 1.00. Corresponding values of depression of the impedance loci ($\gamma$) are indicated in (C).
Fig. 10 A, where \( R_a \) and \( R_b \) are 1.5 and 4.0 kΩ · cm\(^2\), respectively, and where \( C_a \) and \( C_b \) are 1.5 and 20 μF/cm\(^2\), respectively. Although the time constants are sufficiently different at 2.0 Hz (\( R_a C_b \)) and 70.8 Hz (\( R_a C_a \)) to clearly indicate the existence of two membranes in series, ideal separation of the respective (non-depressed) semicircles would require a far greater difference in time constants to yield Nyquist plots that would clearly indicate the exact magnitudes of the \( R_a \) and \( R_{tp} \) and thus the series sum of the magnitudes of \( R_a \) and \( R_b \) on the real axis, \( ReZ_T \). In practice, however, it would be most unusual for \( R_p \) to be infinitely large.

Plotted in Fig. 10 B are normalized impedance loci of the ideal cells (frequency-independent capacitance) shunted by \( R_p \)'s of 50, 10, and 1 kΩ · cm\(^2\) compared to the cells alone (\( R_p = \infty \), Fig. 10 A). Such plots underscore the major influence played by \( R_p \) in determining the appearance of the impedance loci when \( R_p \) is in the range of values of \( R_a \) and \( R_b \). Taken at face value, the plot with \( R_p = 1 \) kΩ · cm\(^2\) could be misinterpreted to indicate that \( R_a \gg R_{tp} \), when in fact this is not the case. Thus, it is critically important to obtain independent estimates of the \( R_p \) before fitting data to models to avoid the possibility of mischaracterizing the behavior of the cells in terms of their fractional transcellular resistances and capacitances.

To the extent that apical and basolateral membranes exhibit dielectric relaxations, analysis and fitting of data additionally require recognition that the impedance semicircles will be depressed (Awaysa et al., 1999). Accordingly, for apical and basolateral membranes, respectively:

\[
Z_a = \frac{R_a^{\text{fit}}}{1 + (j \omega R_a^{\text{fit}} C_a^{\text{fit}})^{\gamma_a^{\text{fit}}}} \quad (12)
\]
\[
Z_b = \frac{R_b^{\text{fit}}}{1 + (j \omega R_b^{\text{fit}} C_b^{\text{fit}})^{\gamma_b^{\text{fit}}}} \quad (13)
\]

and \( Z_T = ((Z_a + Z_b) R_p)/(Z_a + Z_b + R_p) \). Here, as above, formulation of such a model assumes that capacitance will be calculated at single frequencies corresponding to those at the time constants \( R_a^{\text{fit}} C_a^{\text{fit}} \) and \( R_b^{\text{fit}} C_b^{\text{fit}} \). To our knowledge there is no way to determine the frequency dependence of dielectric behavior at either the apical and/or basolateral membrane under these experimental conditions (\( R_a \approx R_b \)). Nevertheless, the existence of \( \alpha \) dispersions would give rise to impedance loci that would require inclusion of power law dependencies (\( \gamma_a^{\text{fit}}, \gamma_b^{\text{fit}} \)) and hence, depressions of the impedance semicircles.

Illustrated in Fig. 11 are depressions of the impedance loci under conditions where \( C_a \) and/or \( C_b \) is complex (\( C_a^*, C_b^* \)). The data were calculated assuming that apical and/or basolateral membrane capacitances exhibit a single \( \alpha \) dis-
The capacitances in parallel with an increase in frequency, as these cannot be fit to models (Eq. 11) that assume an equivalent cellular resistance. When the capacitances are complex, when the capacitance associated with the basolateral membrane dielectric dispersion, indicating clearly that the asymmetric impedance loci shown in (A)originate from the apical membrane dielectric dispersion. For the purpose of illustration we have specifically chosen a relaxation frequency of 110 Hz for the dispersions at 1.5 μF/cm² and C_a = 1.5 μF/cm², indicating the dependence of the impedance locus on frequency. The magnitudes of the respective ideal (nondepressed) semicircles are shown by the thin solid lines that intersect the real axis so that \( R_a + R_p = \Re Z_a \) as frequency approaches zero. (B) Shown are transepithelial impedance loci normalized to their values of \( R_p \) when \( R_p = \infty \), 50 kΩ · cm² and 1 kΩ · cm², indicating the dependence of the impedance locus on \( R_p \) when \( R_p \) is in the range of values of \( R_a \) and \( R_b \).

**Fitting of impedance data**

We have noted above that estimating the cellular resistances and membrane capacitances can be troublesome when the \( R_p \) is finite and in the range of values of the \( R_a \) and \( R_b \) (see Fig. 10 B). For illustration, we have generated impedance data at identical frequencies used in our own experiments and used a least-squares nonlinear curve-fitting routine (Scientist, Micromath Scientific, Salt Lake City, UT) to fit the data over the parameter space of Eq. 1 where \( Z_a \) and \( Z_b \) are defined by Eqs. 12 and 13, respectively. \( R_a \), \( R_b \), and \( R_p \) were 1500, 4000, and 10,000 Ω · cm², respectively. We assumed...
a frequency-independent $C_b = 20.0 \mu F/cm^2$ and an apical membrane $C_a$ with an $\alpha$ dispersion centered at 110 Hz like the one described above. The Nyquist plot of the data points and the fitted line are shown in Fig. 12. Although the fitted line is not perfect at frequencies above $\sim 324$ Hz (Fig. 12 B), the errors in estimation of the $R_a$ ($R_a^{fit} = 1528 \Omega \cdot cm^2$), $R_b$ ($R_b^{fit} = 4014 \Omega \cdot cm^2$), and $R_p$ ($R_p^{fit} = 9857 \Omega \cdot cm^2$) are $< -1.5\%$. As expected, the $\gamma_a^{fit}$ was depressed (0.915) while the $\gamma_b^{fit}$ was not depressed (1.00). $C_a^{fit}$ at 1.97 Hz was 20.11 $\mu F/cm^2$. $C_a^{fit}$ at 83.9 Hz was 1.24 $\mu F/cm^2$ and was identical in value to the $|C_a^{dc}|$ of the $\alpha$ dispersion at this frequency. If we assumed additionally that both $C_a$ and $C_b$ were complex, and the data so generated (not shown) were fit as above, the errors in estimation of the $R_a$ ($R_a^{fit} = 1568 \Omega \cdot cm^2$), $R_b$ ($R_b^{fit} = 4012 \Omega \cdot cm^2$), and $R_p$ ($R_p^{fit} = 9795 \Omega \cdot cm^2$) were $< -4.5\%$. $\gamma_a^{fit}$ was depressed (0.912) and the $\gamma_b^{fit}$ was also depressed (0.987). $C_a^{fit}$ at 1.98 Hz was 20.0 $\mu F/cm^2$ compared to the $|C_a^{dc}|$ of 19.5 $\mu F/cm^2$. $C_a^{fit}$ at 82.9 Hz was 1.23 $\mu F/cm^2$ and was identical in value to the $|C_a^{dc}|$ of the $\alpha$ dispersion at this frequency. Thus, to a good approximation, and despite frequency dependence of capacitance, the $R_a$, $R_b$, and $R_p$ can be determined with reasonable certainty. However, the capacitances are constrained to those at specific frequencies dictated not only by the $R_a$ and $R_b$, but also by $R_p$. Accordingly, in the face of changes of $R_a$, $R_b$, and/or $R_p$, changes of $C_a^{fit}$ and/or $C_b^{fit}$ cannot be attributed only to changes of membrane area when membranes exhibit dielectric dispersions. Indeed, because changes of capacitance can occur in the absence of change of membrane area through change of relaxation frequencies and/or dielectric increments at any frequency (Awady et al., 1999), independent methods are required to assure that changes of capacitance reflect change of membrane area.

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FIGURE 11  Solid lines show depressions of the transepithelial impedance locus when $C_a$ is complex ($C_a^*$ (A), when $C_a$ is complex ($C_a^*$ (B), and when both $C_a$ and $C_b$ are complex (C). Dashed lines show the impedance locus when $C_a$ (1.5 $\mu F/cm^2$) and $C_b$ (20.0 $\mu F/cm^2$) are independent of frequency. $R_a = 10,000 \Omega \cdot cm^2$, $R_b = 1500 \Omega \cdot cm^2$, $R_p = 4000 \Omega \cdot cm^2$. $C_a^*$ ($C_a^{fit} = 1.5 \mu F/cm^2$, $C_a = 0.9 \mu F/cm^2$, $f^a = 110$ Hz, $\alpha_a = 0.6$). $C_b^*$ ($C_b^{fit} = 20.0 \mu F/cm^2$, $C_b = 12.0 \mu F/cm^2$, $f^b = 110$ Hz, $\alpha_b = 0.6$).

FIGURE 12 Transepithelial impedance data (0.244–200 Hz) were subjected to nonlinear curve-fitting to a model of apical and basolateral membrane depressed semicircles (see text) (A). $R_a = 10,000 \Omega \cdot cm^2$, $R_b = 1500 \Omega \cdot cm^2$, $R_p = 4000 \Omega \cdot cm^2$. $C_a^*$ ($C_a^{fit} = 1.5 \mu F/cm^2$, $C_a = 0.9 \mu F/cm^2$, $f^a = 110$ Hz, $\alpha_a = 0.6$). $C_b = 20.0 \mu F/cm^2$. Note deviation of fitted line from the actual data points at frequencies >324 Hz (B).
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REFERENCES


