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Dielectric Properties of Epithelial Monolayer Cultured on Planar Permeable Support

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Mardin-Darby canine kidney (MDCK) cells were cultured on membrane filters. The cells formed monolayers, which were subjected to admittance measurements over a frequency range 0.1 kHz to 10 MHz. A single dielectric dispersion was found around 1 kHz, being analyzed based on a simple equivalent circuit model composed of the cell monolayer capacitance and conductance and a series conductance for the aqueous medium. Mean value of the monolayer capacitance was 1.8 $\mu F cm^{-2}$. The monolayer conductance was 1~10 $mScm^{-2}$, which was sensitive to Ca^{2+} in the bathing solution of the cell monolayer. The removal of Ca^{2+} from the basal solution increased the monolayer conductance by about ten times, the change being reversible by the addition of Ca^{2+} into the basal solution. On the other hand, Ca^{2+} in the apical solution had no effect on the monolayer conductance. This suggests that the opening and closing of the intercellular junctions are regulated by Ca^{2+} levels of the basal solution.

KEY WORDS: Epithelial monolayer/ Intercellular junction/ Ca^{2+}/ Monolayer capacitance/ Monolayer conductance

INTRODUCTION

Cultured epithelial cell layers offer a good model system for studying the mechanism of epithelial transport. MDCK cells derived from dog kidney form monolayers on permeable planar supports. The cell-monolayer showed a transport function similar to natural epithelia of kidney[1,2]. In the present study, we carried out dielectric analysis of the MDCK-cell monolayer to characterize its structural and electrical properties related to the transport function.

MATERIALS AND METHODS

Mardin-Darby canine kidney (MDCK) cells were cultured on membrane filters (Millipore HAWP 01300) in Dulbecco's modified Eagle medium (DMEM) supplemented with 100 mg/l kanamysin and 5% fetal calf serum. The cells formed a confluent monolayer on the membrane filter after 1 day culture. The monolayer was mounted between two lucite chambers having a platinized Pt electrode attached to a brass block as a water jacket (see Fig. 1) in order to measure admittance across the monolayer. Admittance measurements were carried out with an HP 4192A Impedance Analyzer over a frequency range 10 Hz to 10 MHz.

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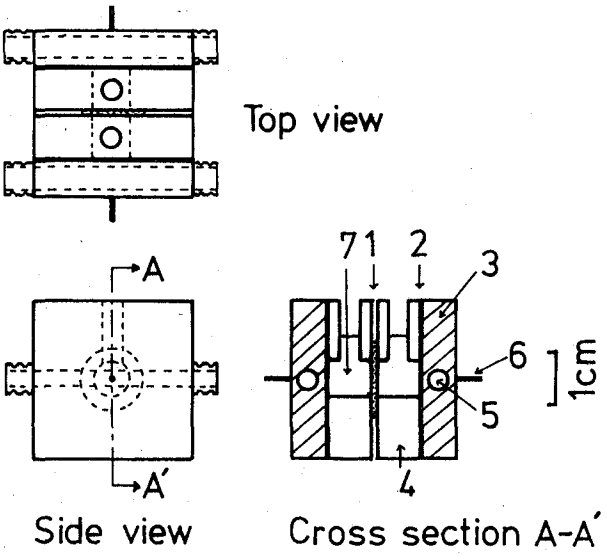

Fig. 1. A thermostated Ussing type cell used for dielectric measurements. 1, cell monolayer with membrane filter; 2, Pt electrode; 3, brass block; 4, lucite spacer; 5, circulating water; 6, connecting lead; 7, bathing solution.

RESULTS AND DISCUSSION

Dielectric dispersion of cell monolayers

Figure 2 shows frequency dependence of the capacitance (C_t) and conductance

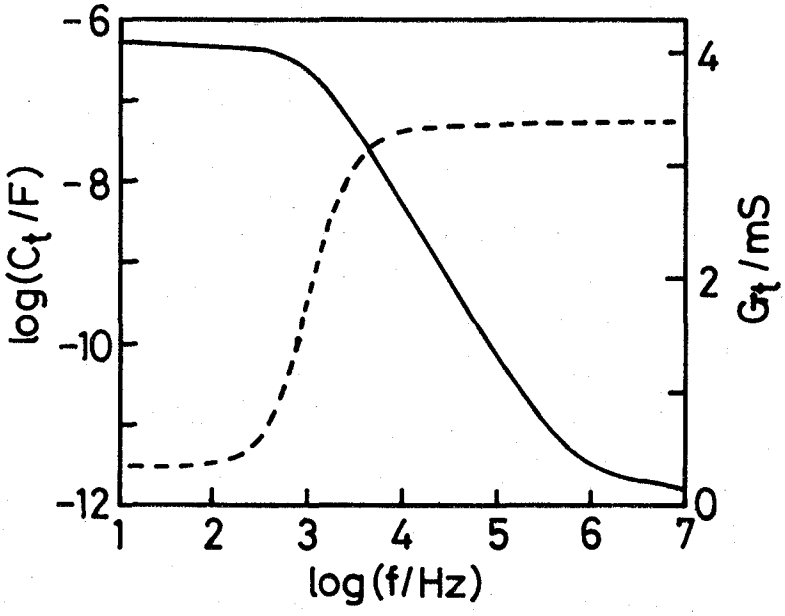

Fig. 2. Dielectric dispersion of MDCK-cell monolayer measured in DMEM at 37°C

(G_t) of a MDCK-cell monolayer including bathing solutions (fetal-free DMEM with 10 mM Hepes-NaOH, pH 7.4). A single dielectric dispersion was found around 1 kHz and the Cole-Cole plots traced a semicircle with a slightly depressed center (Fig. 3).

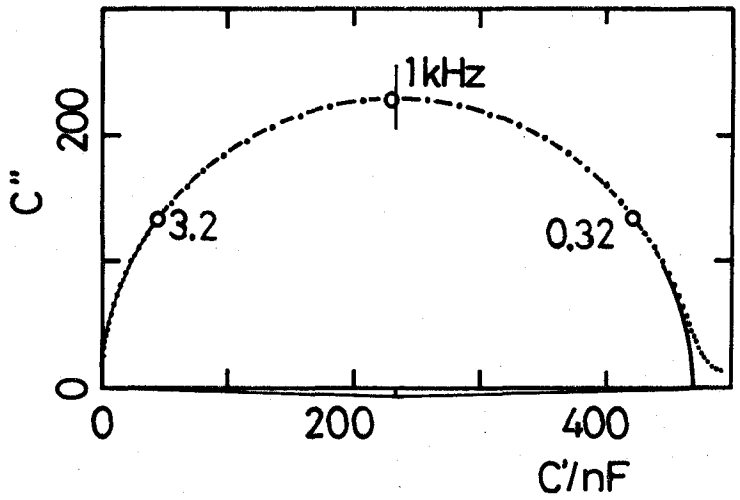

Fig. 3. Cole-Cole plots of the data in Fig. 2.

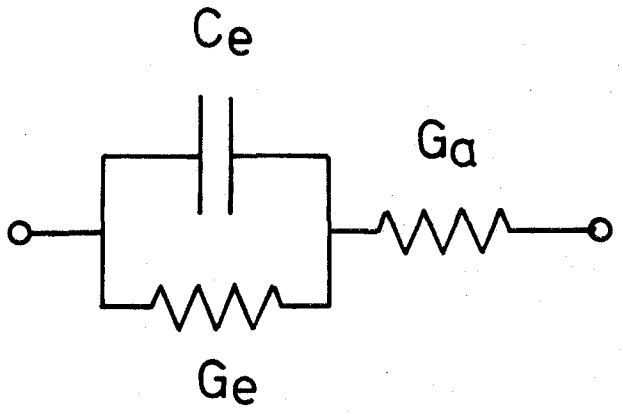

Fig. 4. An equivalent electrical circuit for a MDCK-cell monolayer including bathing solutions. C_e, cell monolayer capacitance; G_e, cell monolayer conductance; G_a, series conductance for the bathing solutions.

Since the cell monolayer-bathing solution system is represented by a simple equivalent circuit model including the monolayer capacitance C_e and conductance G_e and a series conductance G_a for the bathing solutions (Fig. 4), the capacitance C_t and conductance G_t of the system are given by

$$C_t = \frac{C_t}{1+(\omega\tau)^2}\ , \qquad (1)$$

$$G_t = G_l + \frac{(G_h - G_l)(\omega\tau)^2}{1+(\omega\tau)^2}, \tag{2}$$

where $\omega = 2\pi f$, f is frequency, and the dielectric parameters, C_l, G_l, G_h and τ are as follows:

$$C_l = C_e \frac{G_a^2}{(G_e+G_a)^2}, \tag{3}$$

$$G_l = \frac{G_e G_a}{G_e+G_a}, \tag{4}$$

$$G_h = G_a, \tag{5}$$

$$\tau = \frac{1}{2\pi f_0} = \frac{C_e}{G_e+G_a}. \tag{6}$$

Rearranging eqs. (3) and (4), the monolayer capacitance C_e and conductance G_e are given by

$$C_e = C_l \frac{(G_l+G_a)^2}{G_a^2} = C_l \frac{(G_l+G_h)^2}{G_h^2}, \tag{7}$$

$$G_e = \frac{G_l G_a}{G_a - G_l} = \frac{G_l G_h}{G_h - G_l}. \tag{8}$$

The monolayer capacitance was calculated from eq.(7) to be 1.8 μFcm^{-2}, being in good agreement with that obtained by Cereijido et al.[3] from dc transient measurements. The monolayer conductance was found to be 1–10 mScm^{-2}.

Effect of Ca^{2+} on monolayer conductance

In general, cell monolayer conductance depends on a paracellular shunt conductance corresponding to intercellular junctions, which are modulated by Ca^{2+} in bathing solutions. The MDCK-cell monolayer has two surfaces: the one is the basal surface attached to the membrane filter and the other is the apical surface. Since the two surfaces differ in structure, Ca^{2+} in the apical and basal solutions are expected to have different effects on the intercellular junctions.

By replacing the apical solution (fetal-free DMEM) with Ca^{2+}-free saline containing 2.5 mM EGTA, the monolayer conductance G_e remained constant (Fig. 5). On the other hand, under the reverse condition (i.e., the apical solution contains

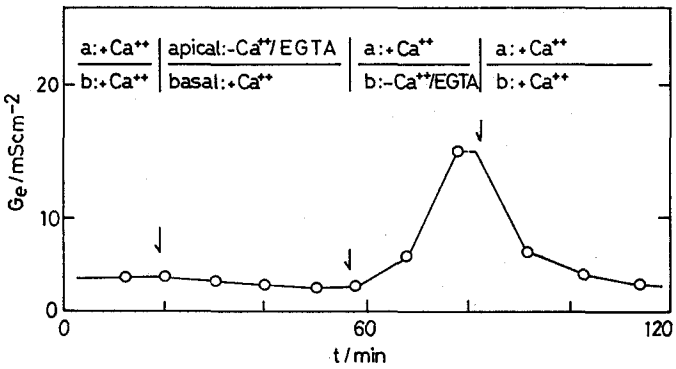

Fig. 5. Effect of Ca^{2+} on monolayer conductance. The bathing solutions were changed at the points indicated by allows.

Ca^{2+} and the basal one is Ca^{2+}-free), G_e increased up to about 10 times the control value within 20 min. The value of G_e returned to the control level by replacing the basal solution with a Ca^{2+} containing medium. This suggests that the opening and closing of the intercellular junctions are regulated by Ca^{2+} levels of the basal solution.

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