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Electrical Properties of Cultured Epithelioid Cells (MDCK)

E. Stefani and M. Cereijido

Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Dept. of Physiology and Biophysics, Apartado Postal 14-740, México 14, D.F., Código 07000

Summary. This is a study of the intracellular electrical potential, membrane resistance, and capacity of MDCK cells (epithelioid of renal origin) cultured in monolayers on a collagen couch. These monolayers have a transepithelial resistance of 256 ± 12 (22) ohm cm^2 (mean $\pm$ standard error, and number of observations), and the cells have 61.6 ± 6.3 (92) MΩ across their plasma membrane. The electrical capacity of the cells is 45.1 ± 2.9 (63) pF and is much higher than expected for a cell of its size (diameter 14 μm, height 5 μm) and cannot be attributed to intercellular coupling, as no evidence of this type of connection was found in 20 pairs of neighboring cells. On the contrary, the high capacity is in keeping with previous studies using electron microscopy showing microvilli and a high degree of lateral infolding. The relationship between resistance and capacity was 1981 ± 177 (61) Ω·μF. The cells have an intracellular potential of -40.5 ± 15 (120) mV. Yet the shape of the distribution curve suggests that the actual value may be somewhat higher (some −50 mV). The current/voltage curve shows a marked asymmetry, and in some cells the voltage becomes time-dependent for large, depolarizing current pulses.

Key Words epithelia · tissue culture · MDCK monolayers · membrane potential · membrane resistance · membrane capacity · cell coupling · amiloride

Introduction

MDCK cells can be cultured in monolayers which resemble natural epithelia. In previous studies we have obtained information on the overall electrical properties (Cereijido, Robbins, Dolan, Rotunno & Sabatini, 1978; Cereijido, Rotunno, Robbins & Sabatini, 1978) as well as on the characteristics of the intercellular space limited by occluding junctions (Cereijido, Stefani & Martínez-Palomo, 1980; Martínez Palomo, Meza, Beaty & Cereijido, 1980; Meza, Ibarra, Sabanero, Martínez-Palomo & Cereijido, 1980; Cereijido, Meza & Martínez-Palomo, 1981). In order to complement the electrical characterization of this preparation, in the present article we report data obtained with intracellular electrodes, which give information on the plasma membrane on the MDCK cell.

The monolayer of MDCK cells has an electrical resistance across of some 80 to 300 Ω cm^2 and this, together with the type of discrimination it exerts between cations and anions, and among univalent cations, suggested that the preparation constitutes a leaky epithelium (Misfeldt, Hamamoto & Pitelka, 1976; Cereijido, Robbins et al., 1978; Cereijido, Rotunno et al., 1978). Yet while in natural leaky epithelia, like the *Necturus* gallbladder, cells are surrounded by an intercellular space with a permeability evenly distributed all along its perimeter, (Cereijido, Chávez & Stefani, 1982), the space between two MDCK cells is essentially tight, but studded with spots of high permeability (Cereijido et al., 1980*b* & 1981). The possibility that it was a tight epithelium agrees with the fact that this monolayer is tight enough to retain water and form blisters (Leighton, Brada, Estes & Justh, 1969; Rabito, Tchao, Valentich & Leighton, 1978; Lever, 1979; Cereijido, Ehrenfeld, Meza & Martínez-Palomo, 1980; Rabito & Tchao, 1980; Lever, 1981) and that under certain culturing conditions it was observed to have a transepithelial electrical resistance of 4,000 Ω cm^2 or more (Simmons, 1981). However, it is pertinent to take into account that the concepts of "leakiness" and "tightness" are not necessarily dependent on the value of the overall electrical resistance. Thus the salivary gland has a relatively low transepithelial resistance due to an unusually high chloride conductance, yet its paracellular route is quite tight (Augustus, Bijman, van Os & Slegers, 1977). The study of the electrical resistance across the cell membrane of MDCK cell may help to clarify these points.

Furthermore, the electrical properties of epithelial cells, as well as their metabolic cooperativity, depend on the existence of intercellular coupling (*see* Loewenstein, 1981). Therefore, we also explore the possibility of cell-cell coupling between MDCK cells.

Materials and Methods

Cell Culture

Starter MDCK cultures were obtained from the American Type Culture Collection (MDCK, CCL-34) (Madin & Darby, 1958). In most experiments cells were between 56 to 68 passages. Cells were grown at 36.5 °C in roller bottles with an air-5% CO_2 atmosphere and 100 ml of Complete Dulbecco's Minimal Essential Medium (CMEM) with Earle's salts [Grand Island Biological Co. (GIBCO) 430-1,600, Grand Island, N.Y.], 100 U/ml of penicilin and 100 μ/ml of streptomycin, and 10% calf serum (GIBCO 617). Cells were harvested with trypsin-EDTA (GIBCO 540), and plated at confluence on glass coverslips coated with rat-tail collagen and contained in petri dishes. They were kept at 36.5 °C in an air-5% CO_2 atmosphere with constant humidity (V.I.P. CO_2 incubator 417, Lab Line Instruments, Inc., New Brunswick, N.J.). The cells were also plated on disks of a nylon cloth coated with collagen as described by Cereijido, Robbins et al. (1978). These disks were mounted as a flat sheet between two Lucite® chambers with CMEM to measure the electrical resistance across the whole monolayer. This was done by recording the voltage deflection elicited by a pulse of 100 μA cm^2. The resistance of the support and solutions was subtracted.

Electrophysiology

Microelectrode recordings were made in monolayers cultured on glass coverslips coated with collagen on the stage of a Leitz phase contrast microscope. The recording solution was CMEM. Experiments were carried out at 37 °C. The bath temperature was electronically controlled and was monitored with a small tip YSI Telethermometer located close to the culture coverslip. To avoid evaporation the saline was covered with a thin layer of oil (Cannon Instruments Co.). Microelectrodes were pulled with a two-stage puller (P77 Brown Flaming, Sutter Inst. Co.), from microfiber borosilicate glass capillary tubes (Hilgenberg, OD 1.0 mm; ID 0.5 mm). They were filled with 3 M KCl or 4 M K-acetate. We preferred microelectrodes filled with 4 M K-acetate since they had less rectification. Microelectrode resistances ranged from 60 to 250 MΩ. In most cases we used microelectrodes with resistance at 100 to 150 MΩ which gave stable impalements and showed little rectification properties with the currents we used (<0.5 μA). The microelectrodes used had a small tip potential (0 to 10 mV) and if its properties checked after the impalement were altered, the recording was discarded. Microelectrodes were connected to standard high impedance amplifiers (WPI KS 700) which allowed us to record the membrane potential and inject current simultaneously via a subtracting circuit. The injected current was recorded with a WPI preamplifier (KS 700) or via a current-to-voltage converter (Analog Devices 52) with 100 MΩ in the negative feedback loop, thus $1\ mV \equiv -0.01$ nA. The bath was grounded via a chlorided silver wire which was connected to the negative input of the current-to-voltage converter.

Prior to penetration the voltage subtracting circuit was carefully adjusted delivering current steps (0.1 to 1 nA). In addition, the input capacity corresponding to the microelectrode and the amplifier was adjusted with a feedback circuit built in the amplifier. Micropipettes which showed prominent rectification were discarded as this stage. Once the cell was impaled, occasional slight adjustment of the balance was needed and the null point could be accurately determined because of the relatively long time constant of the membrane in comparison with the time constant of the recording circuit. This procedure could be optimally carried out with the negative capacity compensation set close before oscillation. On several occasions the same cell was impaled with two separate microelectrodes (Fig. 7) confirming the adequacy of the procedure. Furthermore, the null point was rechecked after withdrawal of the micropipette from the cells. Square pulses of current of increasing amplitudes were delivered to obtain the current/voltage relationship. The membrane time constant was measured at 63% of the steady-state level of the voltage response.

The input resistance of the cell was taken as the ratio between the voltage response and the corresponding injected square pulse of current. Measurements were performed at the end of 0.1 to 0.5 nA pulses with a duration of 20 to 50 msec. The input capacity was taken constant and the input resistance. Since, as will be shown in Results, the cell reacts isopotentially for small negative pulses of current, the membrane resistance can be expressed for convenience as *ohm* times *microfarads*, each microfarad representing in turn a certain amount of membrane area (generally 1 cm^2).

The microelectrodes were mounted on piezoelectric step drive (Burleigh, Inchworm Controller, PZ-550) mounted on a Huxley type micromanipulator. To impale the cells, 4-μm steps were used. Occasionally brief oscillations produced by the capacity compensation circuit helped the penetrations.

To investigate electrical coupling between cells, two adjacent cells were simultaneously impaled with two duplicated recording and injecting set-ups.

Results

As described above, square pulses of current were continuously delivered from the tip of the microelectrode, so a successful impalement was detected by: a) a sharp deflection of the electrode potential; b) obtainment of a new steady (intracellular) potential, and c) the appearance of a large voltage deflection elicited by the pulse of current. Impalements were of two different types: 1) those where the jump in potential as well as the electrical resistance attained a constant value from the beginning of the impalement, and 2) those where the potential and the resistance rose and achieved a steady value in one or two minutes (Fig. 1). Provided that the microelectrode resistance was above some 50 MΩ, the potential never fell to a lower value; therefore, we used two groups of microelectrodes: one had 92 ± 10 (18) MΩ before beveling and the other 169 ± 43 (7). Both gave similar values of membrane potentials and resistances. Beveling of the tip of the microelectrode did not make an obvious difference and was used only in a few impalements. The use of a pneumatic table was essential to avoid spontaneous jumps of the microelectrodes out of the cell.

Further indications of the stability of the values recorded are given in Figs. 4 and 5, where the series of voltage deflections in both directions superimposed on the screen of the storage oscilloscope start each time from exactly the same baseline. The criteria for accepting a given penetration were: a)

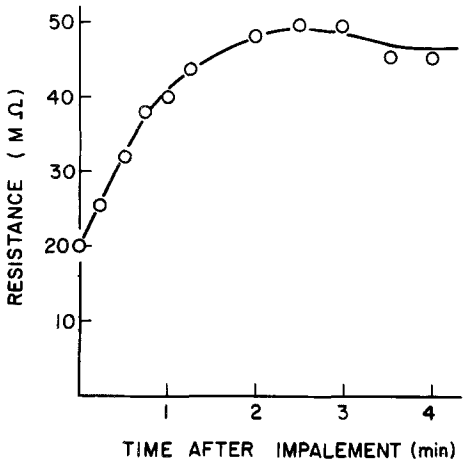

Fig. 1. Time course of the effective electrical resistance as recorded with a microelectrode of 99 MΩ and negative current pulses of 0.1 nA and 30 msec. The MDCK cell was at passage 83 and was plated 7 days earlier. The recording was made in CMEM at 36 °C. Notice that the effective resistance increases and reaches a plateau some 2 min after impalement

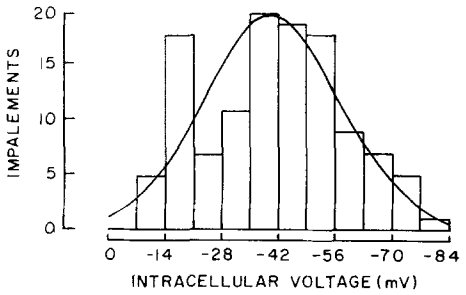

Fig. 2. Histogram of resting potentials

that voltage and resistance were steady; b) this value was not below the maximal voltage deflection obtained in that penetration; c) upon withdrawal the voltage line returned to zero; and d) the resistance of the microelectrode had not changed. However, leak around the microelectrode insertion might not be neglected. Thus the distribution of the membrane potentials recorded (Fig. 2) shows that most values tend to form a normal bell, but there is a group with a peak around −19 mV that may correspond to leaky impalement. Therefore, the value of the membrane potential shown in the Table might be an underestimation, and the higher values of the distribution shown in Fig. 2 should be considered (*see* Kuffler & Vaughan-Williams, 1953).

Using small current pulses of around 0.1 nA which do not cause rectification (*see below*) the electrical resistance was 61.6 MΩ, the voltage deflection reached −40 mV, and followed a simple exponential (Fig. 3) as for a simple *R/S* circuit in

Table. Electrical parameters of MDCK cells cultured in confluent monolayers on glass coverslips

Resting potential (mV)	−40.5 ± 1.5	(120)
Resistance (MΩ)	61.6 ± 6.3	(92)
Capacity (pF)	45.1 ± 2.9	(63)
Resistance (ΩμF)	1,981 ± 177	(61)
Intercellular coupling (and number of cell pairs explored)	0	(20)
Resistance across the monolayer (Ωcm^2)[a]	256 ± 13	(22)

[a] The resistance across the monolayer was measured in monolayers of MDCK cells cultured on a nylon cloth coated with collagen. The current pulses used were of 100 μA cm^{-2}. The contributions of the support and bathing media were subtracted.

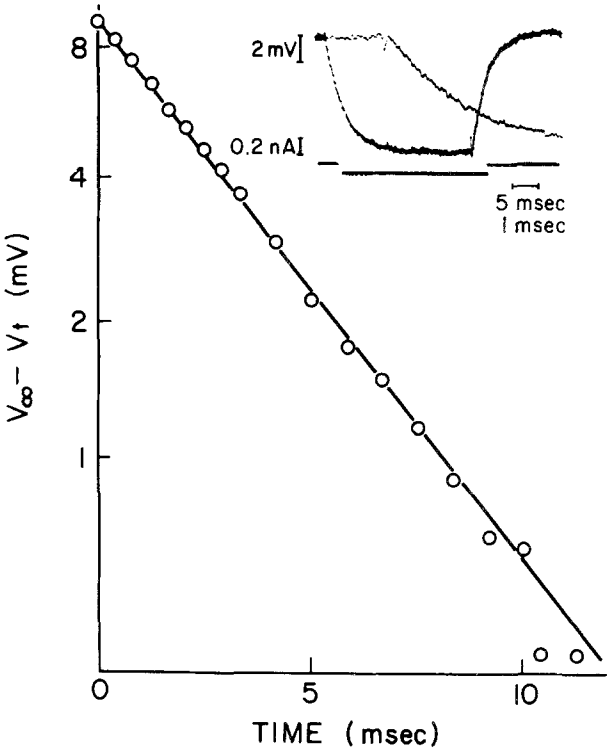

Fig. 3. Measurement of electrical parameters using a small negative pulse. The voltage deflection, recorded at two different sweep speeds, was elicited by a −0.1 nA pulse. From the semilog plot a time constant of 3.6 msec was calculated. The initial resistance of the microelectrode was 95 MΩ, and was beveled to 49 mΩ. The effective resistance of the cell was 83 MΩ, and the normalized resistance was 3,230 ΩμF. The cells were of passage 83 and were impaled 7 days after plating

parallel. Therefore, the cell reacted as an isopotential sphere. The input capacity (45.1 pF; Table) appears much higher than expected on the basis of the size of the cells (some 7 pF, *see* Discussion). Figure 4 shows the voltage/current curve obtained by passing short current pulses (insert: upper left). There is a clear asymmetry of the voltage response,

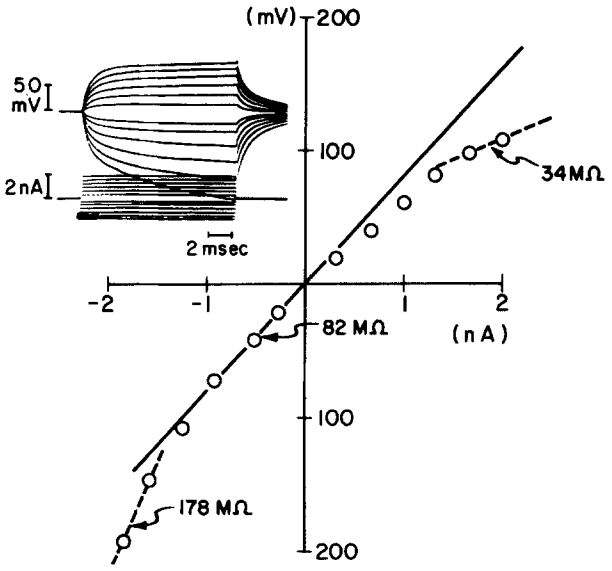

Fig. 4. Voltage/current relationship obtained with current pulses of opposite polarity and various intensities. Notice the asymmetry of the voltage response for large current pulses. The graph shows the voltage/current relationship measured at the end of the pulse. The increase in resistance for large negative pulses is underestimated because the voltage deflections have not reached a steady-state value. Microelectrode resistance: 80 MΩ. Cells were of passage 73 and were plated 4 days before

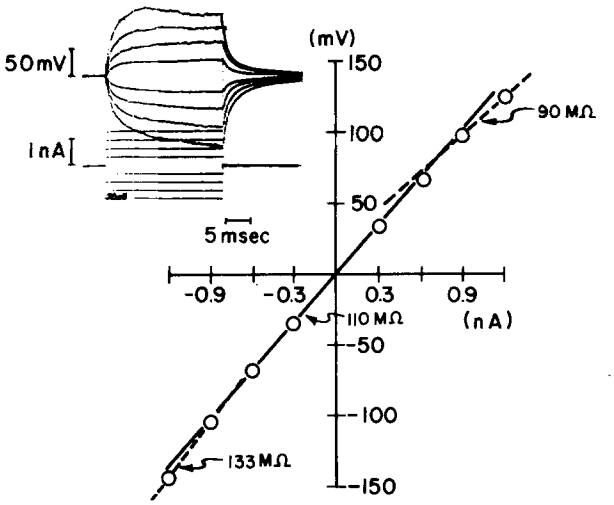

Fig. 5. Time-dependent resistance decrease during large positive pulses. Voltage/current relationship was obtained as in Fig. 4. Notice the reduction of the amplitude of the largest voltage pulse during the square current pulse. Microelectrode resistance 140 MΩ. Cells were of passage 74 and were plated 4 days before

and the resistance varies from 178 to 34 MΩ. In some cells the voltage response for large depolarizing current pulses becomes time dependent (Fig. 5). This time dependence may be accentuated by the use of blockers of Na^+ channels like amiloride (Fig. 6). Nevertheless, in all cases, for the small

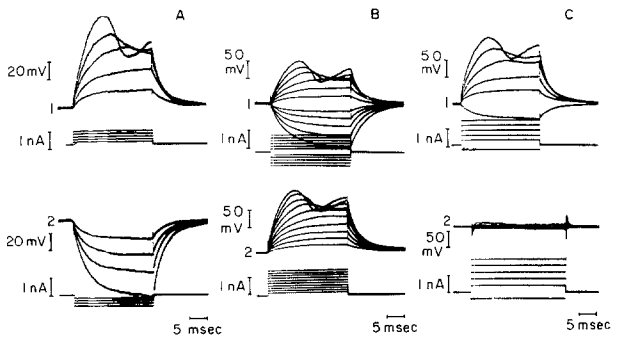

Fig. 6. Time-dependent resistance decrease during large positive pulses in the presence of 10^{-4} M amiloride. In A_1, A_2 and B_1 the membrane potential was −42 mV; in B_2 and C_1 it was −79 mV. In C_2 the microelectrode was pulled out of the cell and a similar series of pulses was delivered. The noisy, but nonsignificant variations obtained in C_2, insure that those in the rest of this Figure were not due to voltage changes at the tip of the microelectrode. The recordings were made in a monolayer of 4 days with a microelectrode of 150 MΩ

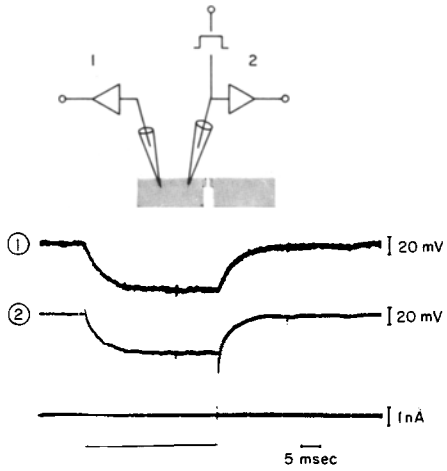

Fig. 7. Impalement of the same MDCK cell with two separated microelectrodes. The upper scheme illustrates the experimental arrangement. Records 1 and 2 were obtained by microelectrodes 1 and 2, respectively, when current was injected via microelectrode 2. The resistances of the microelectrodes were 75 and 80 MΩ, respectively. The cells were of passage 83 and were plated 4 days before

pulses of 0.1 nA used to obtain the values listed in the Table the I/V relationship is linear.

Our study of the electrical properties of MDCK cells included also an exploration of intercellular coupling. For this purpose we impaled a given cell with a microelectrode, injected a square current pulse, and with a second microelectrode impaled in neighbor cells we explored whether the current pulse was detectable. Figure 7 shows that when the two microelectrodes were inside the same cell, the pulse injected through one (microelectrode No. 2 of Fig. 7) was sensed by both of them, and that

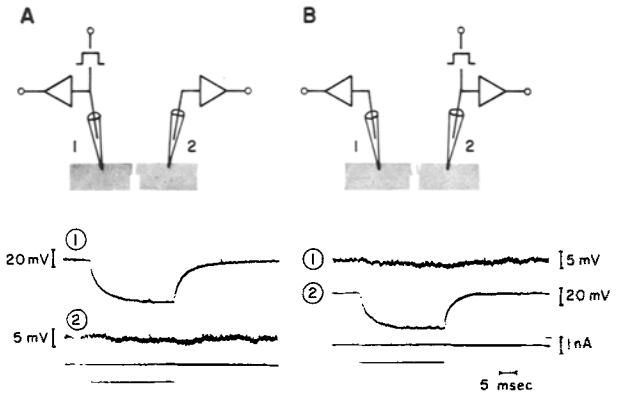

Fig. 8. Impalement of two adjacent MDCK cells. The upper schemes illustrate the experimental arrangement. Records 1 and 2 were obtained with microelectrodes 1 and 2, respectively. The resistance of the microelectrodes was around 90 MΩ in both of them. Notice the large gain in records *A*2 (*left*) and *B*1 (*right*). Cells as in Fig. 7

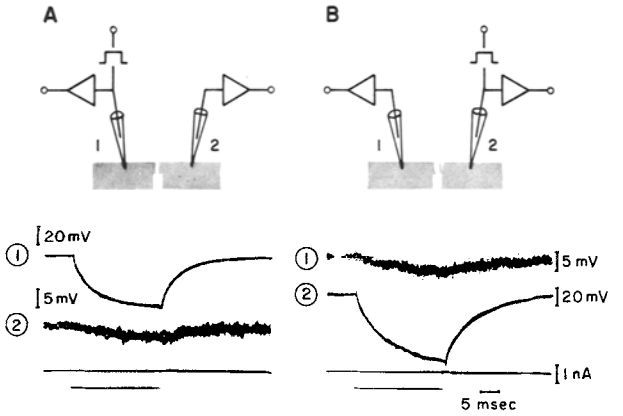

Fig. 9. Impalement of two adjacent MDCK cells. Details as in Fig. 8. Notice the existence of some small degree of electrical coupling between the cells as shown in records 1 and 2

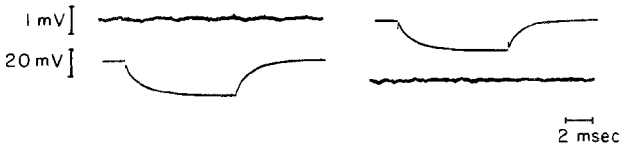

Fig. 10. Impalement of two adjacent MDCK cells as in Fig. 9. In the series of experiments illustrated by this case the record corresponding to the neighbor cell is an average of 256 pulses

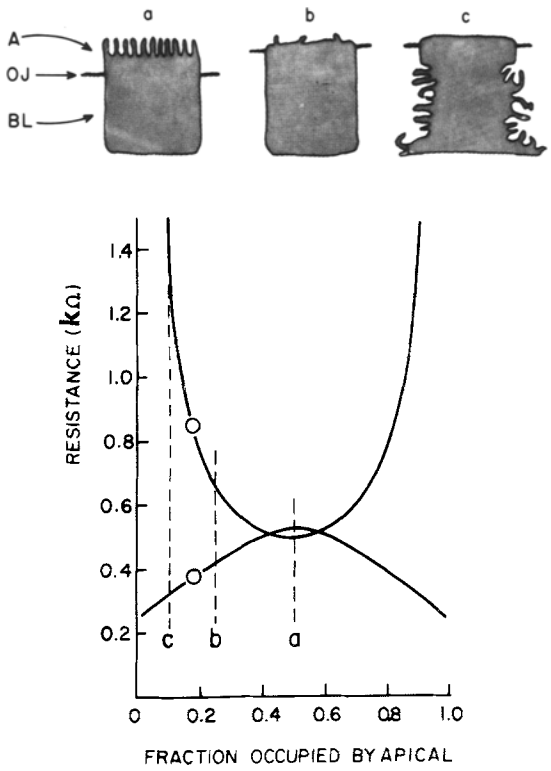

Fig. 11. Effect of the position of the occluding junction (*OJ*) on the electrical resistance of the transcellular and of the paracellular permeation routes assuming a membrane 62 MΩ per cell and 256 Ωcm^2 across the whole monolayer (Table). *Above*: The plasma membrane of the cell is divided into an apical (*A*) and a basolateral (*BL*) region occupying; (*a*) 50% each; (*b*) 1:4, and (*c*) 1:10, respectively. *Below*: Theoretical curves correspondings to the contribution of the transcellular (upper) and the paracellular (lower) resistance to the overall resistance of a monolayer with 5×10^5 cells per square centimeter. Dashed lines correspond to the resistance of the routes in the examples given at the top. The two circles correspond to the electrical resistance through the transcellular and the paracellular route of an MDCK cell with an apical region occupying 0.17% of the total plasma membrane, as suggested by measurements performed on electron micrographs

the size of the signals were quite similar (−44 and −42 mV). However, when the microelectrodes were in neighboring cells (Fig. 8, left) the current-injecting microelectrode (number 1) sensed the voltage variation, but the exploring microelectrode (number 2) did not. For this second recording the sensitivity of the oscilloscope was increased (5 mV per division instead of 20 mV) lest the signal be much smaller. Optically it is easy to insure that the second microelectrode was on the neighbor cell, but in order to insure that the absence of signal was not due to misplacement of the exploring microelectrode, we checked that it was in fact inside the cytoplasm by passing the current pulse through this microelectrode, and trying to detect it with the first (Fig. 8, right). Therefore, both microelectrodes were able to detect the voltage deflection of the cell they impaled, but not that of the neighboring one. We explored 20 pairs of MDCK cells and we have never found a significant degree of coupling. In a few cases we obtained records like the ones displayed in Fig. 9, where the trace of the exploring microelectrode appeared noisy and perturbed, suggesting the presence of a masked

signal. In order to investigate this possibility we recorded and averaged 256 signals of the exploring microelectrode (Fig. 10) and increased the sensitivity of the oscilloscope to 1 mV per division, but we detected no signal. The absence of intercellular connection agrees with the time course of the voltage deflections elicited by square pulses of current (e.g. Fig. 3) indicating that the cell reacts as a single compartment. Should the cells be connected, one would have obtained Bessel functions instead of simple exponential curves.

The Table summarizes the values of the different electrical parameters studied.

Discussion

The mean value of the intracellular potential recorded was −40.5 mV, but the consideration that leaks around the microelectrode, mechanical damage of the cell due to vibration of the tip, and perturbations due to the handling of the preparation may only decrease ionic asymmetries and membrane resistance, suggest that the higher values recorded may reflect more closely the actual membrane potential. Also, if the peak on the left side in Fig. 2 reflects artifactually low values and were neglected, the mean value would shift to some −50 mV. The values obtained compare with those reported for other epithelial cells: −36 mV in rabbit intestine (Rose & Schultz, 1971); −40 to −74 mV in rat intestine (Okada, Sato & Inouye, 1975). It is much higher than the −9.7 to −23.6 mV obtained by Wondergem (1981) in rat hepatocytes in primary monolayer culture. Morphological studies (Valentich, 1981) and immunological tests (Herzlinger, Easton & Ojakian, 1982) suggest that MDCK may be derived from the distal tubule of the kidney. This portion of the nephron has (in the rat) a membrane potential of −61 to −90 mV cytoplasm negative to blood (Windhager & Giebisch, 1965; Wright, 1971; Khuri, Agulian & Kalloghlian, 1972; Malnic & Giebisch, 1972; Wiederholt, Agulian & Khuri, 1974). Therefore, the intracellular potential we found in MDCK cells compares to those in natural and cultured epithelia. Yet the information available at present for this preparation is not sufficient to discuss voltage and conductance in relation to ionic distributions.

An MDCK cell occupies some 200 μm^2 and is some 5 μm high. So if it were smooth it should have a plasma membrane of *ca.* 480 μm^2. Since a typical plasma membrane has a capacity of around one microfarad per square centimeter (Cereijido & Rotunno, 1970) one would expect that an MDCK cell would have some 5 pF. On this basis the actual value found (41.1 pF) appears to be too high. However, MDCK cells have microvilli on the apical region and a high degree of infolding on the lateral cell membrane (Cereijido, Robbins et al., 1978; Cereijido, Rotunno et al., 1978; Cereijido, Ehrenfeld et al., 1980).

Following the pioneer observations of Weidmann (1952), Furshpan and Potter (1959) and Loewenstein and Kanno (1964) that cells can be linked by pathways traversed by ions and large hydrophilic molecules, such junctions were found in a large variety of tissues throughout the phylogenetic scale (for a review *see* Loewenstein, 1981). They appear to be conserved even in cultured epithelia (Flagg-Newton & Loewenstein, 1979; Ledbetter & Lubin, 1979; Atkinson et al., 1981). However, in MDCK cells cultured under the conditions described in Materials and Methods we were unable to detect cell-to-cell communication. This agrees with studies of freeze-fracture electron microscopy which found no indication of gap junctions (Cereijido, Robbins et al., 1978; Cereijido, Rotunno et al., 1978; Cereijido, Ehrenfeld et al., 1980). Therefore, microvilli together with the high degree of infolding, and not the cell-to-cell coupling seem to be responsible for the high value of the capacity.

We may now reconsider whether the monolayer of MDCK cells acts as a truly leaky epithelium. Figure 11 (top) represents cells with a membrane resistance homogeneously distributed over the entire cell membrane, and where the position of the occluding junction determines the fractions of the cell membrane occupied by the apical (F_a) and the basolateral (F_b) regions.

$$F_a + F_b = 1. \tag{1}$$

The resistance of each region will be inversely related to the fraction of the total cell membrane it occupies, and the resistance of the transcellular route of a single cell (R_{CTC}) will be the sum of the resistance of these regions

$$R_{CTC} = \frac{R_m}{F_a} + \frac{R_m}{F_b}. \tag{2}$$

Combining Eqs. (1) and (2) we obtain the parabola

$$R_{CTC} = \frac{R_m}{F_a - F_a^2}. \tag{3}$$

This equation relates the electrical resistance of the transcellular route (R_{CTC}) to the fraction of cell membrane occupied by the apical region (F_a). The upper curve in Fig. 11 was drawn with Eq. (3) assuming a R_m of 62 MΩ (Table). The upper drawing (Fig. 10*a*) illustrates the simplest case: each region

constitutes 50% of the membrane, and the transcellular route presents the minimum of resistance: 248 MΩ. Any departure from this situation can only increase the resistance, either because the apical becomes rate limiting, or else because it occupies more than 50% but then the basolateral becomes rate limiting.

A typical monolayer of MDCK cells has some 5×10^5 cells per square centimeter. If each of these cells offers a resistance of 248 MΩ, the transcellular route ($R_{\text{transcellular}}$) of the entire population will have 496 Ωcm^2. The total resistance across the monolayer ($R_{\text{monolayer}}$) is given by the sum of the resistance of the transcellular (R_{TC}) and the paracellular route in the following manner:

$$\frac{1}{R_{\text{monolayer}}} = \frac{1}{R_{\text{transcellular}}} + \frac{1}{R_{\text{paracellular}}}. \quad (4)$$

Taking $R_{\text{monolayer}}$ as 256 Ωcm^2 (Table) and $R_{\text{transcellular}}$ as 496 Ωcm^2, the resistance of the paracellular route will be 529 Ωcm^2. So, in this hypothetical case, the paracellular route would carry one-half of the current. But actually the apical occupies much less than 50%, because the basolateral is more extensive and has a high degree of infolding. Measurements of the perimeter performed on electron micrographs viewing the cells from the side as the drawings in the upper part of Fig. 11, indicate that the contour of the basolateral is 2.93 ± 0.45 (11) times more extensive than that of the apical. To the best of our knowledge there is no morphometric equation available to transform these linear measurements of the profiles of the cell membrane into the area of the apical and basolateral of an MDCK. Yet, since there are only 3 (out of 5) sides of the basolateral region appearing (two lateral and one basal side), it seems appropriate to multiply the apical/basolateral ratio measured (2.93) by 5/3, to obtain 4.9 (i.e. the area of the basolateral would be 4.9 times larger than that of the apical, and F_a would be 0.17). According to Eq. (3) under such condition the transcellular route of a single MDCK cell will have 430 MΩ, and the 5×10^5 cells per square centimeter would present a resistance of 840 Ωcm^2. According to Eq. (4), in this case the monolayer will have a paracellular route of 370 Ωcm^2 and will be traversed by 70% of the current. It might be pertinent to stress that this is a minimum estimate arising from the assumption that the apical and the basolateral would have identical conductance. Since this is very unlikely, and any other arrangement will confer a higher resistance to the transcellular route, the paracellular route will carry more than 70% of the current.

In summary, as described in the Introduction, in a first series of studies we characterized the overall electrical properties of the monolayer of MDCK cells, and in a second we explored the properties of the paracellular permeation route. In the present article we report information on the cells themselves. As expected, their parameters have the typical values of cells in transporting epithelia, yet they do not present cell-to-cell coupling as many epithelia do. In the last part of the Discussion we compare the resistance across the apical and the basolateral membranes of the whole population of cells (5×10^5 per cm^2) with that offered by a square centimeter of monolayer, but we cannot go beyond conjecture because of the unknown proportion of the cell membrane occupied by the apical and the basolateral regions.

MDCK cell can be plated to form epithelia-like monolayers which acquire pumps, channels and occluding junctions in a few hours. The data here presented, which were obtained in monolayers in steady state (i.e. plated for more than 2 to 3 days) should serve as a starting point to explore such processes through their contribution to the conductance of the cell.

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References

Atkinson, M.M., Menko, A.S., Johnson, R.G., Sheppard, J.R., Sheridan, J.D. 1981. Rapid and reversible reduction in junctional permeability in cells infected with a temperature-sensitive mutant of avian sarcoma virus. *J. Cell Biol.* **91**:573–578

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**:657–658

Cereijido, M., Stefani, E., Chávez de Ramirez, B. 1982. Occluding junction in the *Necturus* gallbladder. *J. Membrane Biol.* **70**:15–25

Cereijido, M., Robbins, E.S., Dolan, W.J., Rotunno, C.A., Sabatini, D.D. 1978. Polarized monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* **77**:853–880

Cereijido, M., Ehrenfeld, J., Meza, I., Martínez-Palomo, A. 1980. Structural and functional membrane polarity in cultured monolayers of MDCK cells. *J. Membrane Biol.* **52**:147–159

Cereijido, M., Meza, I., Martínez-Palomo, A. 1981. Occluding junctions in cultured epithelial monolayers. *Am. J. Physiol.* **240**:C96–C102

Cereijido, M., Rotunno, C.A. 1970. Introduction to the Study of Biological Membranes. Gordon and Breach, London

Cereijido, M., Rotunno, C.A., Robbins, E.S., Sabatini, D.D. 1978. Polarized epithelial membranes produced *in vitro*. *In*: Membrane Transport Processes. J.F. Hoffman, editor. Vol. 1, pp. 433–461. Raven Press, New York

Cereijido, M., Stefani, E., Martínez-Palomo, A. 1980. Occluding junctions in a cultured transporting epithelium: Structural and functional heterogeneity. *J. Membrane Biol.* **53**:19–32

Flagg-Newton, J.L., Loewenstein, W.R. 1979. Experimental depression of junctional membrane permeability in mammalian cell culture. A study with tracer molecules in the 300 to 800 dalton range. *J. Membrane Biol.* **50**:65–100

Furshpan, E.J., Potter, D.D. 1959. Transmission at the giant motor synapses of crayfish. *J. Physiol. (London)* **145**:289–325

Herzlinger, D.A., Easton, T.G., Ojakian, G.K. 1982. The MDCK epithelial cell line expresses a cell surface antigen of the kidney distal tubule. *J. Cell Biol.* **93**:269–277

Khuri, R.N., Agulian, S.K., Kalloghlian, A. 1972. Intracellular potassium in cells of the distal tubule. *Pfluegers Arch.* **335**:297–308

Kuffler, S.W., Vaughan-Williams, E.M. 1953. Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they inervate. *J. Physiol. (London)* **121**:289–317

Ledbetter, M.L.S., Lubin, M. 1979. Transfer of potassium. A new measure of cell-cell coupling. *J. Cell Biol.* **80**:150–165

Leighton, J., Brada, Z., Estes, L., Justh, G. 11969. Secretory activity and oncogenicity of a cell line (MDCK) derived from canine kidney. *Science* **163**:472–473

Lever, J.E. 1979. Inducers of mammalian cell differentiation stimulate dome formation in a differentiated kidney epithelial cell line (MDCK). *Proc. Natl. Acad. Sci. USA* **76**:1323–1327

Lever, J.E. 1981. Regulation of dome formation in kidney epithelial cell cultures. *Ann. N.Y. Acad. Sci.* **372**:371–381

Loewenstein, W. 1981. Junctional intercellular communication: The cell-to-cell membrane channel. *Physiol. Rev.* **61**:829–912

Loewenstein, W.R., Kanno, Y. 1964. Studies on an epithelial (gland) cell junction. I. Modifications of surface membrane permeability. *J. Cell Biol.* **22**:565–586

Madin, S.H., Darby, N.B. 1958. As catalogued in: American Type Culture Collection Catalog of Strains. **2**:574–576

Malnic, G., Giebisch, G. 1972. Some electrical properties of distal tubular epithelium in the rat. *Am. J. Physiol.* **223**:797–808

Martínez-Palomo, A., Meza, I., Beaty, G., Cereijido, M. 1980. Experimental modulation of occluding junctions in a cultured transport epithelium. *J. Cell Biol.* **87**:736–745

Meza, I., Ibarra, G., Sabanero, M., Martínez-Palomo, A., Cereijido, M. 1980. Occluding junctions and cytoskeletal components in a cultured transporting epithelium. *J. Cell Biol.* **87**:746–754

Misfeldt, D.S., Hamamoto, S.T., Pitelka, D.K. 1976. Transepithelial transport in cell culture. *Proc. Natl. Acad. Sci. USA* **73**:1212–1216

Okada, Y., Sato, T., Inouye, A. 1975. Effects of potassium ions and sodium ions on membrane potential of epithelial cells in rat duodenum. *Biochim. Biophys. Acta* **413**:104–115

Rabito, C.A., Tchao, R. 1980. [^{3}H] ouabain binding during the monolayer organization and cell cycle in MDCK cells. *Am. J. Physiol.* **238**:C43–C48

Rabito, C.A., Tchao, R., Valentich, J., Leighton, J. 1978. Distribution and characteristics of the occluding junctions in a monolayer of a cell line (MDCK) derived from canine kidney. *J. Membrane Biol.* **43**:351–365

Rose, R.C., Schultz, S.G. 1971. Electrical potential profile across rabbit ileum. *J. Gen. Physiol.* **57**:639–663

Simmons, N.L. 1981. Ion transport in tight epithelial monolayers of MDCK cells. *J. Membrane Biol.* **59**:105–114

Valentich, J.D. 1981. Morphological similarities between the dog kidney cell line MDCK and the mammalian cortical collecting tubule. *Ann. N.Y. Acad. Sci.* **372**:384–405

Weidmann, S. 1952. The electrical constants of Purkinje fibres. *J. Physiol. (London)* **118**:348–360

Wiederholt, M., Agulian, S.K., Khuri, R.N. 1974. Intracellular potassium in the distal tubule of the adrenalectomized and aldosterone treated rat. *Pfluegers Arch.* **347**:117–123

Windhager, E.E., Giebisch, G. 1965. Electrophysiology of the Nephron. *Physiol. Rev.* **45**:214–244

Wondergem, R. 1981. Transmembrane potential of rat hepatocytes in primary monolayer culture. *Am. J. Physiol.* **241**:C209–C214

Wright, F.S. 1971. Increasing magnitude of electrical potential along the renal distal tubule. *Am. J. Physiol.* **220**:624–638

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