RESEARCH COMMUNICATIONS

DETERMINATION OF THE IMPEDANCE LOCUS OF RABBIT CORNEAL ENDOTHELIUM

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It has been recently found that the rabbit corneal endothelium generates a potential difference in the order of 0.5 mV across it (inside or aqueous side negative) which appears related to fluid transport across this membrane (Fischbarg, 1972). Several other transporting epithelia (e.g., gall bladder, kidney proximal tubule and ascending limb of Henle's loop, small intestine, choroid plexus) present also relatively high permeabilities and low electrical potentials across them, just as the present one (Fromter and Diamond, 1972; Fischbarg, 1973). In the present communication, the impedance locus and values of electrical resistance and capacitance across corneal endothelium are reported for the first time. These results agree extremely well with evidence from physiological, light and electron microscope observations, and the emerging pattern is interpreted in terms of a proposed model.

Corneas were scraped free of epithelium, and the endothelium, supported by the stromal layer, was mounted in a chamber and bathed with a physiological solution (Dikstein and Maurice, 1972) as previously described (Fischbarg, 1972). Electrical impedance was measured with a four-electrode system (Dennis, 1959). An AC current (10 μA) of varying (2 Hz to 100 kHz) frequency was applied by the two outer electrodes through the entire area of the preparation (2.2 cm^2), and the small AC potential difference across it (10–300 μV) detected with the two inner electrodes was augmented with a tuned AC amplifier and displayed on an oscilloscope. Freshly mounted endothelia had a resistance of 41 ± 5 $\Omega \cdot cm^2$ (SEM, $n = 14$; range: 16–81 $\Omega \cdot cm^2$) and their impedance loci were circular arcs centered below the real axis (Fig. 1). When the endothelial cells were scraped off (leaving only the stroma) the resistance fell to 4–20 $\Omega \cdot cm^2$, and the locus changed to a point on the real axis. The resistance of the fresh preparation decreased with time and eventually

reached the value of the stroma after about 6–8 h. A marked fall in resistance was also obtained with Ca-free solutions. The potential difference was initially 650 ±70 μV (n = 12; range: 250–1,100 μV), and its magnitude kept a correlation with that of the resistance both initially and as a function of time. A cell relaxing agent, cytochalasin B (20 μg/ml), produced a marked change in the impedance locus and in the resistance calculated from it (Fig. 1 *a*); ouabain 10^{-6} M, however, did not affect either impedance locus or resistance (Fig. 1 *b*). The capacitance of the endothelium, measured from the frequencies of maximal reactance (cf. Brown and Kastella, 1965), was 0.41 ±0.03 μF/cm^2 (n = 11). Individual values were all very close (range: 0.31–0.55 μF/cm^2), and did not change either as a function of time or under the effects of cytochalasin B or ouabain.

Some simple models for the electrical behavior of other preparations have been used (e.g., Cole and Cole, 1941; Brown and Kastella, 1965) in conjunction with impedance locus determinations. Presently, several lines of evidence can be tied together in the model of Fig. 2 *b*. Average dimensions found for endothelial cells, intercellular spaces, and terminal bars or gap junctions (Kaye et al., 1972; Kaye and Pappas, 1962; Kaye et al., 1973; Fischbarg, 1973) are given in Fig. 2 *a*; the cell perimeter length per unit area was estimated to be 1,200 cm/cm^2 (cells are hexagonal, 18–20 μm diameter) and the gap junction length 0.25 μm. All spaces were assumed to be filled with 150 mM NaCl, since recent evidence shows penetration of horseradish peroxidase (Kaye et al., 1972) and LaOH (Leuenberger, 1973) across gap junctions and into intercellular spaces. Values thus calculated for the resistance of intercellular spaces (R_S) and gap junctions (R_G) are about 18 $\Omega \cdot$cm^2 and 4 $\Omega \cdot$cm^2, respectively. They fall close to the resistance measured experimentally across the fresh preparation (range: 16–81 $\Omega \cdot$cm^2). If the terminal bars were "tight," this resistance would be expected to approximate values for intact cell membranes (approximately 1,000 $\Omega \cdot$cm^2). On the other hand, if the terminal bars were "leaky," the resistance would be about 18 $\Omega \cdot$cm^2, and values as low as this are actually measured after several hours in vitro. Increasingly "leakier" gap junctions could then underlie this progressive decrease in resistance. Alternative explanations (leaky cell membranes, or progressive falling off of individual cells) are unlikely since intracellular potentials have been recorded from these cells after several hours in vitro (Di Ulio and Edelhauser, personal communication; Fischbarg and Dorfman, unpublished) and no endothelial cells are observed to be missing when monitored with a "specular reflection" (Dikstein and Maurice, 1972) microscope. In support of this model is also the fact that cytochalasin B, which decreases the potential difference across the endothelium (Fischbarg, 1972) and causes profound alterations in cell geometry as seen by transmission and scanning electron microscopy (Kaye et al., 1973) has been found here to decrease the endothelial resistance. Although a direct simultaneous effect on the transport mechanism cannot be excluded, all the evidence strongly suggests that its effects are primarily due to an increased shunt. In contrast, ouabain, which abolishes the endothelial potential difference (Fischbarg, 1972) in some 10 s

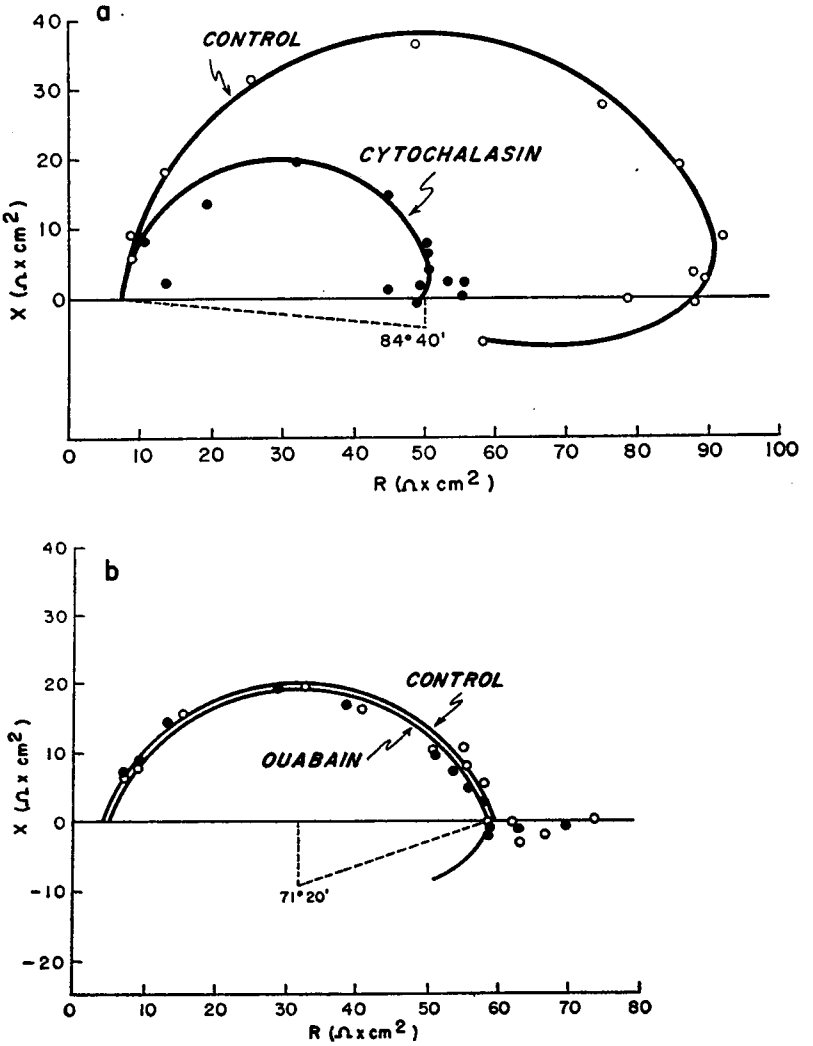

FIGURE 1 A plot of imaginary vs. real components of endothelial impedance results in circular arcs (loci) centered below the real axis. The resistance (in parallel with the capacitance in an equivalent circuit, Fig. 2 *b*) is given by the segment of real axis intercepted by the locus. There was no hydrostatic pressure difference across the preparation. (*a*) Two impedance loci from a representative experiment before and immediately after cytochalasin B 20 μg/ml was added to the inside of the endothelium. (*b*) Two loci before and immediately after 10^{-5} M ouabain is placed on the inside of the endothelium.

(Fischbarg, 1973) but which does not change the electron microscope appearance of intercellular spaces and terminal bars (Kaye et al., 1965), does not change the resistance either (Fig. 1). This suggests that its effect on potential difference is exerted directly on an electrogenic pump (E in Fig. 2).

The scheme presented above suffices to explain the present data remarkably well with only those few assumptions listed. In the same vein, if the capacitance measured is ascribed to the two cell membranes in series (Fig. 2 *b*), the capacitance of each of them comes out to be 0.82 μF/cm^2 in the average. This value is in the range of the

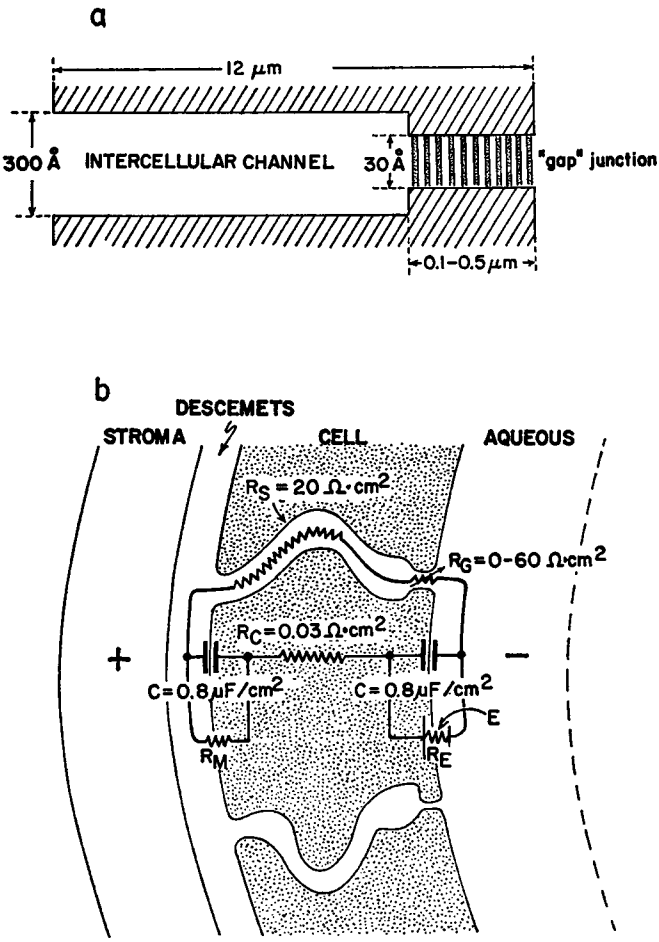

FIGURE 2 (*a*) Average dimensions obtained from electron micrographs are shown in an idealized drawing of the intercellular spaces and terminal bars or gap junctions. Lines inside gap junctions represent electron-opaque structures observed there (Leuenberger, 1973). (*b*) Electrical model superimposed on a schematic drawing of the corneal endothelium. R_S, R_G, R_M, R_C, and R_E: resistance of intercellular spaces, gap junctions, cell membrane, cytoplasm, and ionic pump, respectively. The values indicated were calculated from geometrical dimensions in (*a*) (also see text). The electromotive force E ascribed to the ionic pump is shown for the sake of argument in one likely location (the plasma membrane facing the inside), but alternative locations (such as the lateral plasma membrane) cannot be excluded. C, capacitance of the cell membrane, calculated from the frequencies of maximal reactance.

1 $\mu F/cm^2$ ubiquitously found for cell membranes ever since its first determination in erythrocytes (Fricke, 1926). There is also distinctive evidence of an inductive reactance element such as found for a squid axon membrane (Cole and Baker 1941) and ascribed to nonlinear behavior (Cole, 1947). Finally, it is worth noting that, although concluded on the basis of different evidence, the mechanism of passive ion permeation contemplated here is in general agreement with that recently proposed for the gall bladder (Fromter and Diamond, 1972). The present model also incorporates an electrogenic ionic pump as an important feature for this and perhaps other similar epithelia. A more extended treatment of this work will be presented in a subsequent communication.

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