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Epithelial monolayers: A study object for cell communication

LOEWENSTEIN *et al.*[1-3] showed remarkable cell communication in epithelial cell structures. For additional evidence we investigated electrotonic spread in monolayer cell cultures of freshly explanted chick embryo intestinal epithelium grown on glass by the usual tissue culture techniques. Such a monolayer system is a promising means for study of communication between cells. Advantages are the regular arrangement of cells permitting a mathematical analysis and the possibility of microscopic control during the measurement allowing an accurate knowledge of the position of the measuring and stimulating electrodes.

Monolayer cultures were made by explanting approx. 0.5-mm pieces of 10-day-old chick embryo intestine directly onto cover glasses in Leighton tubes using a medium containing Hanks' balanced saline, 5 % lactalbumin hydrolysate and 20 % calf serum. Within 3 days a large area of continuous epithelium was formed around the explant in which the cells had been *ab origine* in contact with one another. At that time the measurements were carried out.

The microelectrodes (micropipettes filled with 3 M KCl; 40–70 MΩ) were inserted in the cells under the microscope by micromanipulation. The input resistance, *i.e.* the seeming increase of the electrode resistance by the insertion (6–10 MΩ)[3], and the stability of the intracellular potential (about 30 mV) were criteria for successful impalement.

Electrotonic spread was measured by means of one electrode, while injecting through the other electrode a low-frequency square wave current (10 Hz; $1 \cdot 10^{-9}$–$1 \cdot 10^{-8}$ A). The measuring electrode was used as a roving one impaling consecutively cells at different distances from the one containing the stimulating electrode. In this way the complete potential distribution in the monolayer could be determined. Injection and measurement were made with respect to an electrode immersed in the bathing fluid (Hanks solution at 29°).

Analysis. The following assumptions were made: (1) The cell arrangement has a honeycomb structure (Fig. 1). (2) All cells are of equal height (h) and have equal sides (hexagonside d). (3) The contributions of intracellular and extracellular fluid resistances are negligible with respect to the junctional membrane resistance (specific resistance ρ_e ($\Omega \cdot cm^2$)) and to the membrane resistance of the nonjunctional membrane (specific resistance ρ_m ($\Omega \cdot cm^2$)). These resistances are supposed to be independent of the trans-membrane voltages. (4) The cells can be considered as ordered in rings around a central one (order number zero) which contains the stimulating electrode. The nth order ring contains $6n$ cells all of which are supposed to have the same potential V_n. (V_n is the potential caused by the injected current and measured with respect to the intracellular equilibrium potential.)

With the help of these assumptions and Kirchhoff's first law, a relation has been established between successive ring potentials:

$$(2n-1)V_{n-1} + (2n+1)V_{n+1} = \beta\, nV_n$$

$$\beta = 4 + \frac{3}{2}\sqrt{3}\,\frac{\rho_e}{\rho_m}\cdot\frac{d}{h}$$

The ratio V_n/V_0 can be computed now for different values of β (Fig. 2). Measuring results of ratios V_n/V_0 together with estimates of d (5 μm) and h (8 μm) suggest a value $\rho_m/\rho_e \approx 10^2$.

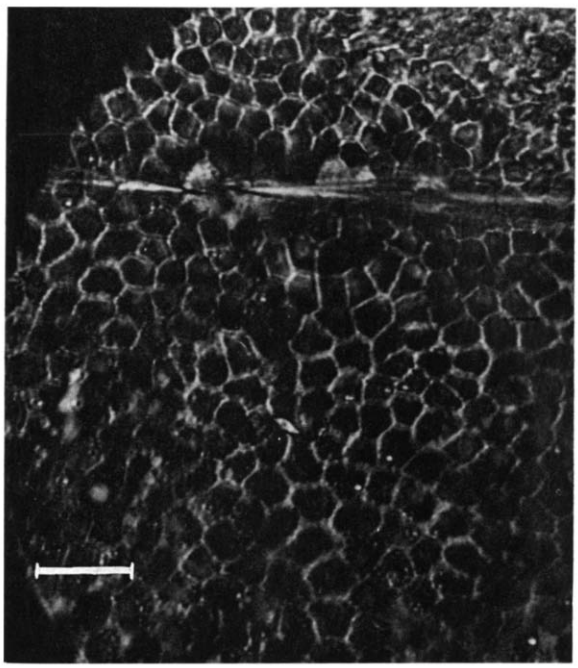

Fig. 1. Monolayer with microelectrodes. Bar = 20 μm.

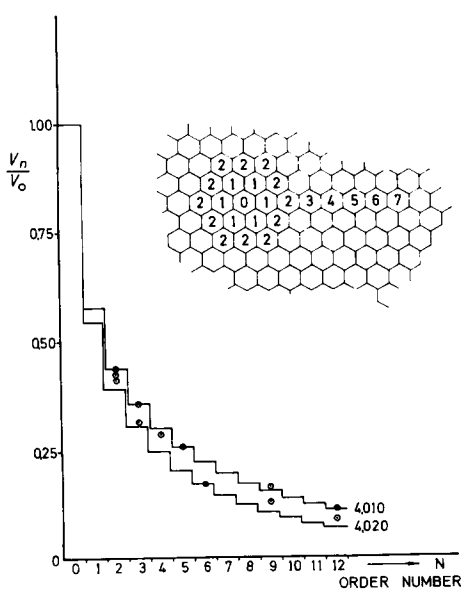

Fig. 2. Electrotonic spread. Solid lines: theoretical values with $\beta = 4.010$ and $\beta = 4.020$, respectively. ⊙, measuring results.

In an equivalent continuous model[4,5] this corresponds to a space constant $\lambda \approx 70\ \mu m$.

Another relation between ρ_m and ρ_e can be derived taking into account the injected current I and the ring voltages V_0 and V_1:

$$I = \frac{6\ d\ h}{\rho_e}(V_0 - V_1) + \frac{3}{2}\sqrt{3}\frac{d^2}{\rho_m}V_0$$

With the help of this relation, the ratio ρ_m/ρ_e and evaluation of the voltage transient following the current injection, absolute values for ρ_m, ρ_e and the specific membrane capacity c_m of the nonjunctional membrane can be estimated. For easier computation the voltage transient was evaluated with the help of the continuous model of electrotonic spread: the monolayer is thought of as a continuum bounded by the nonjunctional membrane, while the junctional membrane resistances are included in the intracellular fluid resistance.

Average results are: $\rho_m \approx 1000\ \Omega \cdot cm^2$; $\rho_e \approx 10\ \Omega \cdot cm^2$; $c_m \approx 10\ \mu F \cdot cm^{-2}$.

The results show a remarkable difference between junctional and nonjunctional resistance. The analysis contains quite a few assumptions but none of them is likely to be responsible for this highly significant difference. This is the more so as assumption (3) is confirmed by the results.

In computing the absolute values of the specific resistances and capacitance, no membrane folding and the like is taken into account. Therefore, the estimated values of the resistances are to be considered as lower limits, the capacitance value as an upper limit. As such they are quite acceptable values.

Besides, the resistance values do not fall outside the range of values computed by others with the help of measurements in contiguous cells. However, we feel that more reliable results may be obtained when the complete potential distribution as induced by the stimulating electrode is evaluated. The influence of local damages and impalements is reduced as many intact cells contribute to the final result.

For studies on cell communication we think this method rather promising as the monolayer, evaluated as a whole with two resistance values and one capacitance value, may be compared with monolayers cultured under different conditions or exposed to special agents.

Finally we want to emphasize that in using monolayers directly from fresh explants and only 3 days in culture the always present risk of introducing artifacts caused by long-term culturing and trypsinization is virtually dismissed[3,7].

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Gas chromatographic determination of the configuration of alanine and serine in staphylococcal cell walls

In 1966, KORMAN[1] reported the presence of small amounts of serine in the cell walls of wild-type strains of staphylococci and elevated levels of this amino acid in mutants isolated from the wild-type strains. TIPPER AND BERMAN[2] have confirmed that there are low but significant amounts of serine in *Staphylococcus aureus* Copenhagen and increased amounts in *S. epidermitis* Texas 26. They have located this amino acid within the pentapeptide cross bridges of the peptidoglycan moiety, and BROWDER *et al.*[3] have established its L-configuration in several strains by microbiological assays.

In the present communication further data are reported on the configuration of the alanine, as well as the serine, in staphylococcal walls as determined by a new gas chromatographic method[4,5]. The procedure, which is based on the use of an asymmetric stationary phase, permits one to resolve and analyze different amino acids conveniently and simultaneously.

TABLE I

MOLAR RATIOS AND CONFIGURATION OF AMINO ACIDS IN STAPHYLOCOCCAL CELL WALLS

The molar ratios were determined on a Beckman-Spinco amino acid analyzer. Configuration was determined by gas chromatography; r = relative retention volume with respect to the corresponding glycine derivative. D/L = molar ratio of the D- to the L-isomer; average of 2–3 determinations.

Strain No.	*Source of reference*	*Amino acids*							
		Lys	*Glu*	*Gly*	*Ala*	*Ser*	*Thr*	*Ile*	*Leu*
NCTC 8511	See ref. 1	1	1.14	4.93	2.91 D/L = 1.90	0.11 L*	Traces	0.10	0.13 §
HS 2123	HS 968 See ref. 1	1	1.02	4.3	3.6 D/L = 2.03	1.0 L**	Traces	Traces	Traces
HS 1159	HS 968	1	1.1	5.10	2.44 D/L = 1.60	0.03 L*	0.004	Traces	Traces
HS 1449	See ref. 1	1	1.08	4.39	2.9 D/L = 2.33	0.70 L*	Traces	Traces	Traces
HS 2136	HS 1449	1	1.1	4.6	2.7 D/L = 1.47	0.28 L*, ***	0.11	0.12	0.16 L§

* Isopropyl ester, $r = 2.16 \pm 0.04$ (standard: D-Ser, 1.98; L-Ser, 2.19).
** Determined by coinjection.
*** Methyl ester, $r = 2.60 \pm 0.03$ (standard: D-Ser, 2.44; L-Ser, 2.63).
§ Methyl ester, $r = 2.22 \pm 0.01$ (standard: D-Leu, 2.08; L-Leu, 2.21).