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ELECTRICAL PROPERTIES OF ISOLATED CELLS OF NECTURUS GASTRIC MUCOSA

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SUMMARY

A micropuncture technique is described for isolated surface epithelial cells and oxyntic cells of Necturus gastric mucosa, and for intact isolated tubules from the same tissue. The potential obtained is similar to that found for the cells in the intact tissue, whereas the measured membrane resistance is about 100 times higher. This is explained by cell–cell coupling in the intact mucosa, and is confirmed by direct two-electrode experiments in the intact tissue or isolated tubule. The calculated tissue resistance is about twice as high as the measured resistance after allowance is made for glandular infolding, hence the possibility remains that there is a low resistance shunt across the tissue.

INTRODUCTION

A description of electrical events on the cellular level associated with transport by the gastric mucosa is complex for the following reasons: The gastric mucosa is a heterogeneous tissue composed of several cell types with different morphological and functional characteristics. Evidence has been presented that these cells contain two or more electromotive forces which may lead to different and independent changes of potential (PD) across mucosal and serosal surface of the plasma membrane[1,2]. In analogy to other epithelial tissues, cells of the same type may be electrically coupled along linear or circumferential pathways, while electrical coupling may or may not exist between different cell types[3]. It is thus apparent that results obtained by placing a microelectrode into a cell of intact mucosa may not give information on independent electrical properties of this cell[4]. Furthermore, such studies do not allow an estimate of the magnitude of intercellular conductive shunts which in analogy to gallbladder[5] and other tissues[6] might constitute major pathways of ion flux across the mucosa. An additional disadvantage of microelectrode studies in intact gastric mucosa is the visual inaccessibility of the gastric glands, which contain the oxyntic cells.

In the present paper, a new method for the study of electrical properties is described, which involved preparation and subsequent micropuncture of isolated intact cells of the gastric mucosa and isolated gastric tubules. The spread of current

Abbreviation: PD, potential difference.

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in oxyntic and surface cell systems is examined by simultaneous measurements in two cells. It may thus be shown that surface and oxyntic cells in intact mucosa are electrically coupled while significant intercellular conductive pathways may also be present. In addition, micropuncture studies in isolated cells may allow an assessment of the electrical properties of specific cell types under a variety of conditions.

METHODS

Isolated Necturus gastric cells were prepared by the pronase technique as previously described[7]. Stripped Necturus gastric mucosa was pinned mucosal surface upwards on the bottom of a lucite dish and shaken in HCO_3^- buffered frog Ringer containing 0.175 % pronase for 120 min. The cell suspension thus obtained was centrifuged at 50 g for 5 min, washed twice in frog Ringer and resuspended either in frog Ringer's solution or in a solution containing 18.5 mM K^+ with correspondingly lower Na^+ levels.

Isolated gastric gland tubules were prepared by a modification of the pronase technique. In this case stripped mucosa was incubated for one hour and then rapidly shaken in pronase solution. Clumps of cells and isolated tubules were readily obtained.

Fig. 1 shows the experimental arrangement used for micropuncture of isolated cells. The cell suspension was placed on the agar filled depression of a microscope culture slide. The cell types were identified under a stereomicroscope. Oxyntic (*i.e.* acid secreting) cells could be differentiated by their size and granular appearance from other cells such as surface cells[7].

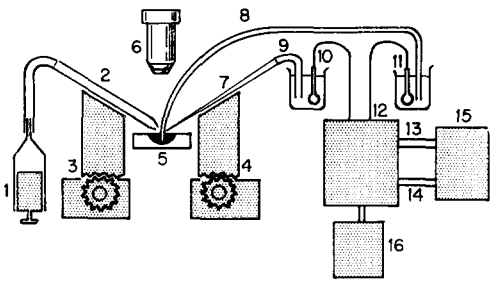


Fig. 1. Experimental arrangement for micropuncture of isolated cells: (1) oil filled syringe; (2) oil filled suction micropipette; (3,4) micromanipulators; (5) agar filled microculture slide; (6) stereomicroscope; (7) microelectrode; (8,9) 3 M KCl–agar bridge; (10,11) calomel electrodes; (12) amplifier; (13,14) recorder connections; (15) recorder or CRO; (16) current source.

For the preparation of microelectrodes, glass capillary tubing was precleaned in chromic acid and alcohol and mounted on a Nastuk electrode puller[8] (Industrial Science Associates) with a 4-mm platinum loop. Microelectrodes were examined microscopically and were filled with 3 M KCl. Their tip resistance was about 50 mΩ and their tip potential was less than 3 mV. As shown in Fig. 1, they were held by a micromanipulator and connected to a WP4 amplifier and thence to a Grass recorder. The amplifier also had provision for sending and monitoring current and a Wheatstone bridge circuit for balancing the electrode response to current before starting a cell puncture.

A micropipette with a tip diameter of approx 5 μm was used for trapping of

isolated cells and immobilizing them during micropuncture. This pipette was also pulled on a Nastuk puller from precleaned capillary tubing. The tip was then broken against the platinum wire of a de Fonbrunnė microforge and fire polished. The micropipette was filled with oil from a Hamilton microsyringe and connected to a suction system, as shown in Fig. 1, care being taken to exclude air bubbles.

When isolated tubules and pieces of intact mucosa were used for obtaining cell potentials, each micromanipulator held a microelectrode. In this way potential recordings could be obtained from 2 cells simultaneously, allowing, for example, current injection into one cell, and recording potential changes in a distant cell.

Micropunctures were considered successful when the measured potential difference remained stable for at least 10 sec. Cell membrane resistance was calculated using Ohm's law from the change in potential produced by a 1–2-sec pulse of 10^{-9} A sent through the intracellular electrode. For obtaining current–voltage curves hyper- and de-polarizing current pulses of varying intensity and 0.5–1-sec duration were sent through the microelectrode, and the change in potential difference plotted against amount of current injected.

Morphological studies were performed on intact mucosae as well as on isolated cells from five animals. After fixation in an ice-cold 1 % solution of OsO_4, veronal acetate buffered to pH 7.4 (ref. 9), the tissues were embedded in Epon. 1-μ-thick sections were cut in an ultramicrotome and stained with toluidine blue, or by the periodic acid–Schiff method. In each of 4 sections from each of 5 animals, the relative surface area of the gastric gland lumina and of the mucosal surface was estimated in intact mucosae by counting the intercepts between these surfaces and the lines of a super-imposed grid (for further details on this method see WEIBEL AND ELIAS[10]).

Additional morphological information was obtained by electron microscopy on thin sections of the same tissues mentioned above. The sections were contrasted with lead hydroxide followed by uranyl acetate and examined in a Philips EM 200 electron microscope at 60 kV. The relative surface area of isolated oxyntic cells were estimated by the method described above, and compared with the surface of smooth spheres of the same diameter.

RESULTS

Potential of isolated cells

Stable PD readings were obtained in about 50 % of the punctures attempted. Failures occurred in cells which disintegrated or swelled while being held by the micropipette or upon puncture by the microelectrode. Cells which appeared swollen prior to puncture or cells with a grossly irregular surface had a PD of less than 5 mV. Further studies, therefore, were confined to cells without morphologic signs of damage such as swelling and irregular surface. Fig. 2 shows a PD recording obtained from an oxyntic cell. The duration of this recording was 20 min. Other recordings were obtained for as long as 60 min with less than 10 % decay in PD. In most cases long-term stability of the PD was not tested, and the electrode was removed from the cell after 1–5 min. In a representative batch of 67 cells the PD ranged from values of about 10 to 90 mV, with a mean ($\pm$ S.E.) of 44 $\pm$ 3 mV (Fig. 3).

In control experiments the PD was determined in surface cells of intact mucosa, and compared to the PD in isolated surface and oxyntic cells. The experimental ar-

rangement was similar to that used for isolated cells (Fig. 1). The mean (± S.E.) PD for the surface cell in the intact mucosa was 43 ± 8 mV. The effect of K^+ concentration change on the PD of isolated oxyntic cells was also measured. When K^+ was increased from 4 to 18.5 mM and Na^+ correspondingly decreased, the PD fell by 17 mV (Table I).

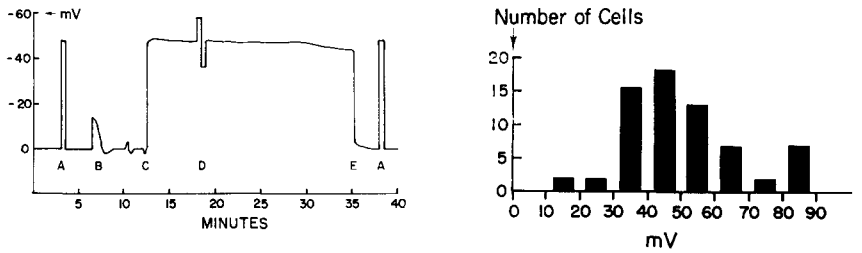


Fig. 2. Cellular potential obtained from oxyntic cell. At A tip resistance is measured, at B current is sent through the electrode and balanced out through the Wheatstone bridge, at C the cell is punctured, at D current is sent in both directions through the electrode, at E the electrode is removed from the cell, and at A tip resistance is checked.

Fig. 3. The distribution of potential difference obtained from a batch of 67 cells.

TABLE I

INTRACELLULAR POTENTIALS

Values in mV ± S.E.

Isolated surface epithelial cells 4 mM K^+	*Isolated oxyntic cells 4 mM K^+*	*Isolated oxyntic cells 18.5 mM K^+*	*Surface epithelial cells in intact mucosae 4 mM K^+*
36 ± 11 N = 12	48 ± 6 N = 22	31 ± 5 N = 11	43 ± 8 N = 15

Resistance of isolated cells

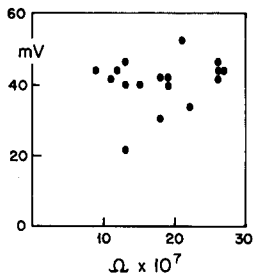
In Fig. 4 the resistance of 17 oxyntic cells is plotted against the measured cell PD. There is no simple relationship between PD and resistance. More detailed resistance studies were carried out as discussed below.

Current voltage curve

Fig. 5 shows current–voltage plots obtained from an isolated surface cell and from a surface cell in intact gastric mucosa, the slope representing the resistance of the system. There is a striking difference in slope between the two experiments. Since in this and all other experiments a uniform and statistically significant curvature ($P < 0.001$) was observed, the slope was calculated from the tangent of a curve which was defined by a cubic equation forced through the origin. The calculated tangents for isolated surface cells, isolated oxyntic cells and surface cells in intact mucosa are given in Fig. 6. Similar values were found for isolated oxyntic cells and surface cells while the mean obtained for isolated surface cells was about 100 times greater than the mean of intact mucosa ($1.5 \pm 0.3 \cdot 10^8$ and $1.6 \pm 0.2 \cdot 10^6 \Omega$, respectively, $P < 0.0001$).

Two electrode system

When stable PD readings were obtained in two surface cells of intact gastric mucosa, a current pulse was applied to one cell and the deflections of PD in both cells were recorded. A PD deflection in the distant cell was used as expression of electrical coupling. An estimate of the type of electrical coupling was obtained by systematic circumferential puncture of surface cells around the cell which contained the current sending electrode. Electrical coupling was found in 10–20 % of the cells with a radial distance of up to 100 μm, *i.e.* about 1–10 cells away from the current sending electrode.



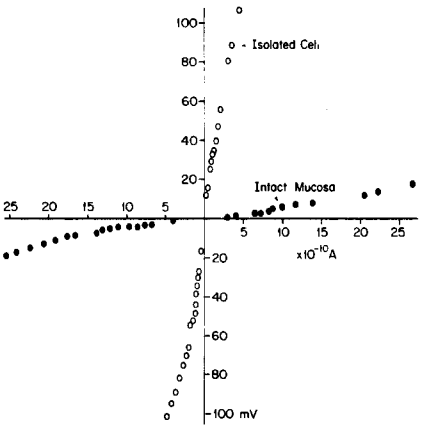
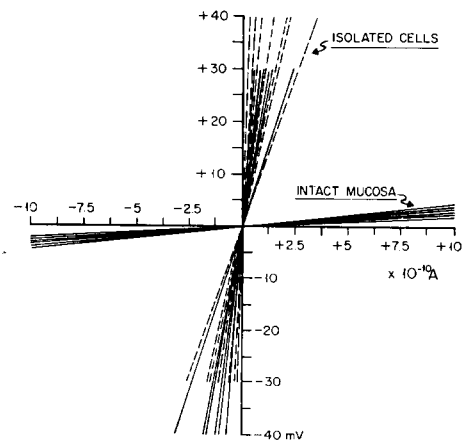


Fig. 4. Resistance of 17 oxyntic cells plotted against PD of the cells showing little correlation.

Fig. 5. Current–voltage plot for the surface epithelial cell in the intact mucosa and when isolated, showing a marked difference in slope, and non-linearity in the depolarization (downward) direction.



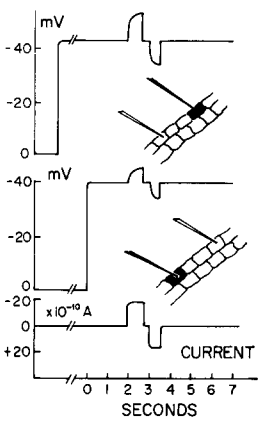


Fig. 6. Calculated tangents to the current–voltage curves obtained for intact mucosa and isolated cells. The tangents were obtained from the equation $y = ax^3 + bx^2 + cx$, solid lines are surface cells, broken lines are oxyntic cells.

Fig. 7. Experiment demonstrating coupling in isolated tubules of Necturus gastric mucosa. The upper electrode is used for recording PD and sending current, and the other electrode records PD changes in another cell. The bottom trace records the amount of current sent.

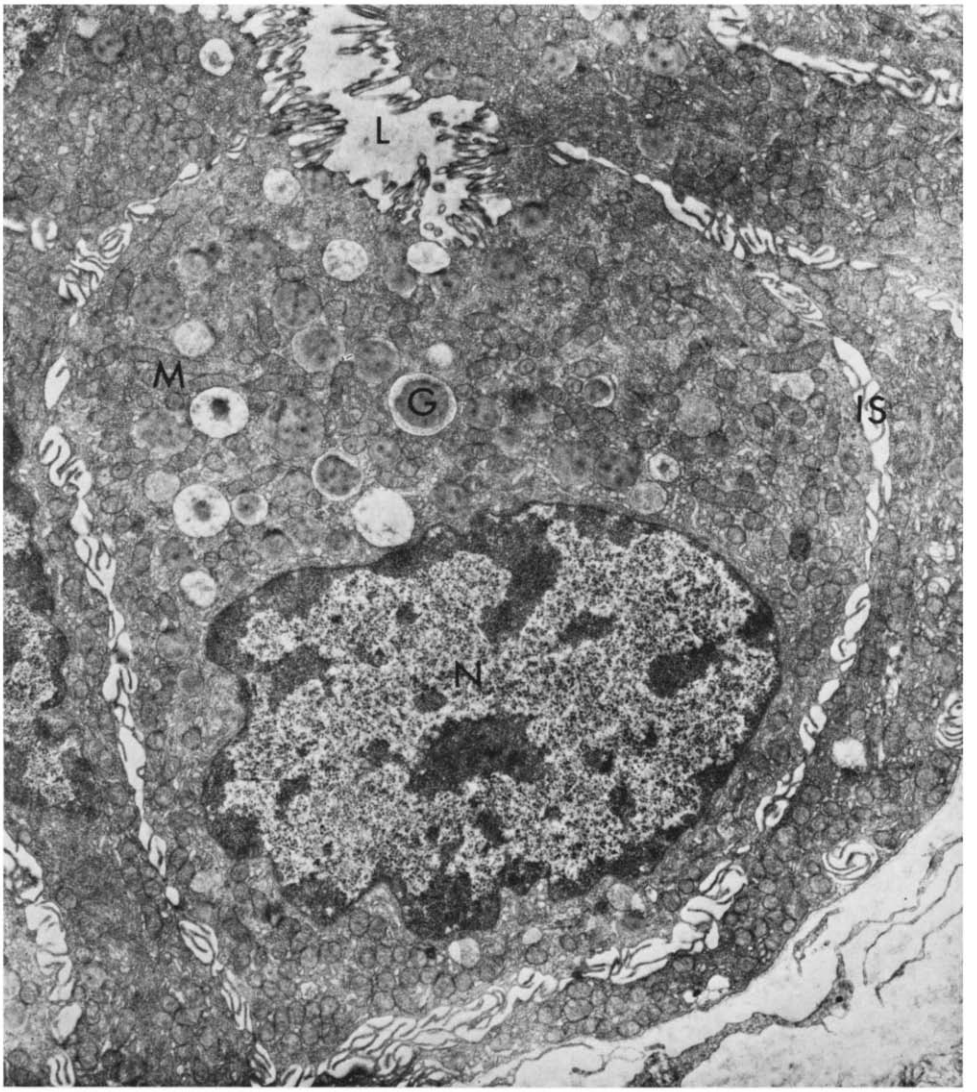


Fig. 8. Electron micrograph of oxyntic cell in gastric mucosa. Numerous microvilli project from the apical cell surface into the lumen (L). Along the lateral and basal cell surfaces similar cytoplasmic projections are seen, extending into the intercellular space (IS). The cytoplasm contains a large number of mitochondria (M) and some secretory granules (G). N, cell nucleus. × 4300.

Electrical coupling between oxyntic cells was examined in isolated gastric tubules. A typical experiment is shown in Fig. 7 demonstrating an attenuation of the current pulse of 50 % over a distance of only 3 cells. In other experiments, the distance for 50 % attenuation was 4–5 cells.

Morphologic observations

The increase in surface area of the mucosa caused by the infoldings of the gastric glands was calculated to be 2.7 ± 0.10 (S.E.) for 5 animals. In the dog the increase

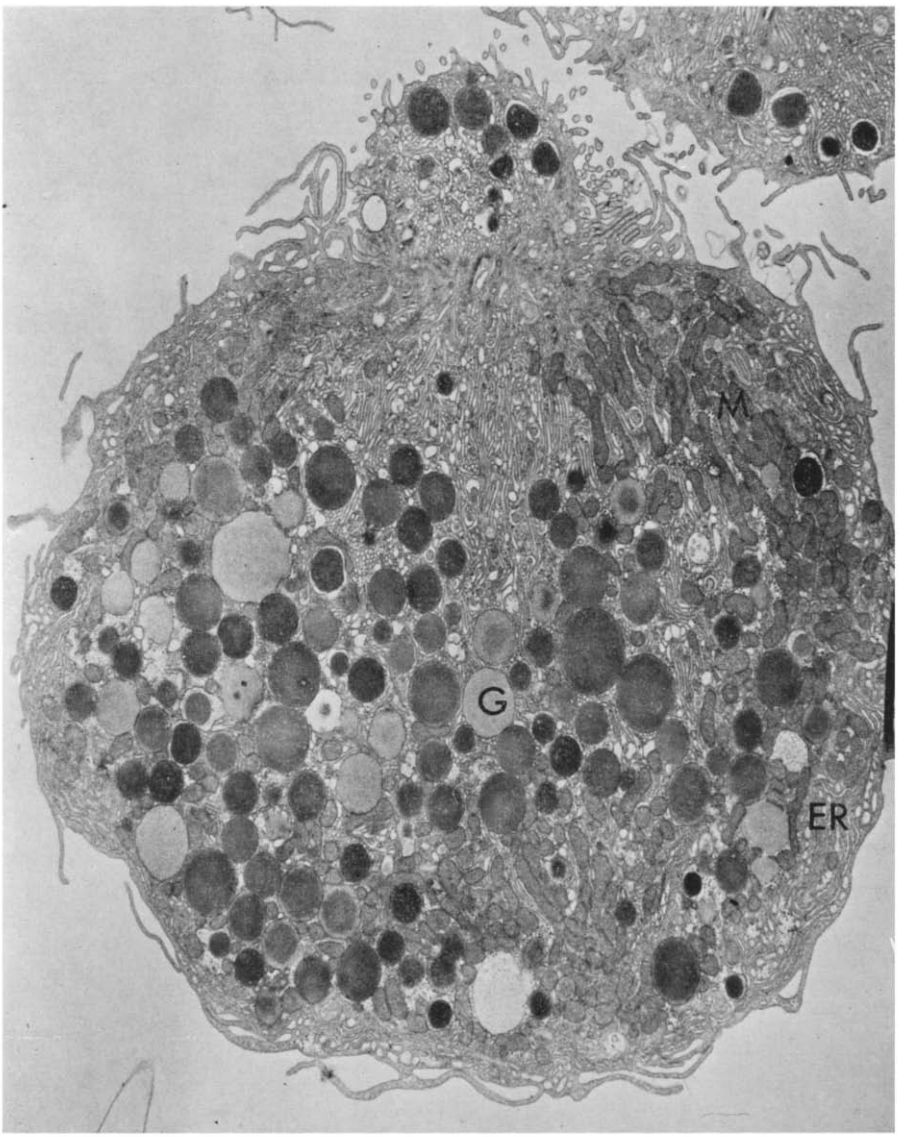


Fig. 9. Electron micrograph of isolated oxyntic cell. The number and size of the microvilli projecting from the cell surface has not changed greatly, as compared to Fig. 8. The general appearance of cytoplasmic components such as the endoplasmic reticulum (ER) and the mitochondria (M) suggest good viability. The nucleus is not included in this section. G, secretory granule. × 4300.

is 13 times (ref. 11). On this basis the mucosal surface of the tissue consists of approx. 37 % surface cells and 63 % oxyntic cells. These values were used for calculation of the specific resistance from values obtained from isolated cells.

Electron micrographs of oxyntic cells from intact mucosa as well as in the isolated state are shown in Figs. 8 and 9. The isolated oxyntic cells showed evidence of good

morphologic integrity and also that there had not been any significant alterations in surface infoldings or microvilli during the isolation procedure. In the isolated oxyntic cells the infoldings and microvilli resulted in a 3–5-fold increase of the surface area as compared to a smooth sphere of the same diameter, whereas for the surface cell this increase amounted to 1.5 times.

DISCUSSION

Potentials of isolated cells

The ability to perform micropunctures in isolated cells allows for the first time a direct assessment of functional properties in specific cell types of gastric mucosa. In the present study, the transmembranal PD of nonstimulated isolated mucosal cells is reported (Table I, Fig. 3). The average PD of these cells is 44 mV and is not significantly different from values obtained from pieces of intact mucosa. It would thus appear that the isolation procedure does not lead to damage of the cell membrane and/or of the mechanisms which are responsible for the PD.

This interpretation should, however, be accepted with some caution since a spuriously normal PD in cells with a damaged plasma membrane could be due to leak of KCl from the microelectrode into the cell. Such a leak would have relatively little effect on the PD of electrically coupled cells of intact mucosa, while in isolated cells its effect would be proportionately much greater. In the present study, the use of fine tip electrodes minimized the occurrence of leakage. A leak is also improbable on the basis of observations with KCl filled microelectrodes in electrically coupled and uncoupled cells of intact toad bladder. If a leak would occur in this preparation, electrical uncoupling of a punctured cell would result in an apparent increase of PD. However, no such increase is observed[3].

The PD of isolated surface cells was not significantly different from the PD of isolated oxyntic cells. Further confirmation of this observation by different techniques is, however, necessary since it has been shown in previous studies that viability of isolated surface cells is lower than of oxyntic cells which in eosin exclusion tests are 80–100 % viable[7].

In previous studies, the K^+ concentration of isolated oxyntic cells as determined by a [^{14}C]inulin method was 67 mM /l cell water (ref. 7). On the basis of this value, the predicted Nernst PD for a perfect K^+ electrode is:

$$\mathrm{PD} = \frac{RT}{nF} \ln \frac{67}{4} = 70 \text{ mV}$$

This value is considerably higher than the PD observed in oxyntic cells, suggesting that the oxyntic cell membrane is permeable to ions other than K^+, such as Cl^- or Na^+. An alternate explanation is that cell injury has occurred, either due to the isolation procedure, or due to the puncture itself. In the latter case, decay of the measured potential from some higher value would be likely to be observed, which was not generally found. Injury due to the isolation procedure is more difficult to exclude, but values of 70 mV have never been observed by us for intracellular potentials even with multiple deep punctures of intact mucosa, where penetration of oxyntic cells is highly probable.

Similar conclusions may be reached from the PD response to change of K^+

concentration in the nutrient solution. A 4.5-fold increase in K^+ leads to a reduction of the PD by 17 mV, while the expected decrease for a membrane with selective permeability for K^+ would be 38 mV. These findings closely correspond to observations in surface cells of intact mucosa[4].

Membrane resistance

The membrane resistance was calculated in all instances from the deflection of the PD in response to a standard current pulse. A more accurate estimate was obtained from the slope of current voltage plots (Figs. 5 and 6). In order to express the resistance per area of cell membrane, the surface area of isolated cells was estimated on the basis of findings in phase contrast microscopy. Thus, isolated surface cell and oxyntic cell are spheres with a radius of 10 μm and 15 μm, respectively, and their resistances are 1800 and 2730 $\Omega \cdot cm^2$, respectively (Table II).

TABLE II

The values obtained for cell dimensions, voltage current slopes and calculated membrane core resistance and space constants for the surface cell, intact mucosa, oxyntic cell and isolated tubule. The values in parentheses are those corrected for infolding of the plasma membrane of the isolated cell as measured in electron micrographs.

	Surface cell		*Oxyntic cell*	
	Intact	*Isolated*	*Tubule*	*Isolated*
Cell dimensions (μm)	30 × 10	$r = 10$	$r = 15$	$r = 15$
V_0/I_0 (Ω)	$1.6 \cdot 10^6$	$1.5 \cdot 10^8$	$7 \cdot 10^6$	$2.0 \cdot 10^8$
R_m ($\Omega \cdot cm^2$)	—	1800 (2700)	—	2730 (8190)
R_c ($\Omega \cdot cm$)	50	—	800	—
Space constant (μ)	1400	—	400	—

Since the relative surface area of isolated oxyntic cells was calculated in electron micrographs to be 3–5 times greater than estimated for a smooth surfaced sphere (lacking microvilli), the estimates for specific membrane resistance are too low by the same factor. In the case of the surface cell, the surface area increase was much less, being about 1.5 times. When these values are used to calculate the specific membrane resistance of the surface cell and oxyntic cell, the values obtained are 2700 and 8190 $\Omega \cdot cm^2$, respectively.

Since the specific membrane resistance of the resting oxyntic cell is considerably higher than that of the surface cell, the larger portion of the tissue conductance in the resting mucosa is accounted for by the conductance of the surface epithelial cell system.

Transmucosal electrical shunts

If such shunts were present, the resistive properties of intact tissue could not be predicted on the basis of findings in isolated cells. In the following section, therefore, predicted and observed resistance of intact mucosa are compared.

Since the resistance R_m of mucosal (or apical) membrane and the resistance R_s of serosal (or basal) membrane in isolated cells are electrically in parallel, the total resistance R_t is:

$$R_t = \frac{R_m \cdot R_s}{R_m + R_s}$$

In the intact mucosa R_m and R_s are electrically in series and thus resistance of the intact mucosa (R) in the Ussing chamber is:

$$R = R_m + R_s$$

If R_m and R_s are assumed to be of equal magnitude, a minimal value for R is obtained which is 4 times the R_t value.

For a mucosa such as the one shown in Fig. 8 whose surface area consists of 63 % oxyntic cell surface and of 37 % surface cell surface, the calculated resistance is approximately:

$$R = 5000\ \Omega \cdot cm^2$$

The observed value of R for intact resting Necturus gastric mucosa in an Ussing chamber is $710 \pm 47\ \Omega \cdot cm^2$ assuming the area is given by the dimensions of the chamber[12]. If the 2.7-fold increase in surface area by the tubular infoldings is taken into account, the corrected observed resistance was $2000\ \Omega \cdot cm^2$. This value can be compared to the value calculated above from isolated cell measurements since in both cases it was assumed the cells had a smooth surface.

It is apparent that the predicted resistance of Necturus gastric mucosa is about twice the observed value. On this basis, therefore, the possibility is not excluded that there are intercellular resistive shunts similar to other tissues such as the gall bladder[5], and the tubule of Necturus kidney[6].

Comparisons of this type would be misleading if the surface area of the mucosal cells would change during isolation. However, by comparing electron micrographs of isolated cells and of cells in the intact mucosae it appeared that there was no major difference in the surface area or the size of the cells under the two conditions.

Another possibility is that there is a qualitative change of the cell membrane as a result of isolation. Gross damage to the membrane is probably excluded by a number of observations such as eosin exclusion, O_2 consumption and good morphology[7]. In addition the cellular Na^+ and K^+ content appears to be the same as for intact tissue[13].

Formation of an altered surface layer, with a resultant increase of resistance is, however, not excluded by these observations. In fact, proteases including pronase have been shown to have stimulatory effects on cell division by initiating escape from contact inhibition[14]. Internal perfusion of squid axon with pronase, however, increased membrane conductance[15]. Since there was no change of surface area upon isolation, the effect of pronase would have to be on the composition of the membrane, to account for the increased resistance. In this process, there can be no alteration of relative K^+ conductance since there was no alteration in the response to extracellular K^+ concentration changes.

Electrical coupling

The resistive properties of isolated surface cells and of surface cells in intact mucosa were examined in current voltage plots (Figs. 5 and 6). As shown by the 100-fold difference in slope, the resistance measured in intact mucosa is much lower than in isolated cells. This difference cannot be explained by a reduction in surface area of the cell membrane during the process of isolation. More likely is the presence of low resistance pathways between cells of intact mucosa which are not present in isolated cells. Such pathways have been described by LOEWENSTEIN AND KANNO[16] who observed electrical coupling between epithelial cells and the movement of fluorescent dyes across cell junctions. This low resistance R_c is electrically in parallel with the membrane resistance R_m with respect to an intracellular current source and is — in the present study — about 100 times smaller than R_m.

Since even in close proximity to the surface cell which receives current pulses only a small proportion of the cells show signs of electrical coupling, circumferential spread of current is very unlikely. A more likely model for the type of coupling assumes radial spread of current along a "cell cable" with a low core resistance R_c and a high resistance of the insulating membrane sheath R_m. For such a cable LOEWENSTEIN AND KANNO[16] derived the following equation:

$$R_m = \lambda c \frac{V_0}{I_0} \tanh \frac{L}{\lambda} \quad (1)$$

where λ is the space constant and is defined by $\lambda = \sqrt{r_m/r_i}$, r_m is the resistance of unit length of the insulating membrane sheath in Ω/cm, r_i is the resistance per unit length of the core conductor in Ω/cm, c is the circumference of the "cell cable", V_0 is the value of potential difference at the current source, I_0 is the total current flowing into the core from the internal electrode, and L is the total length of the core conductor. For surface cells of intact tissue, c is $80 \cdot 10^{-4}$ Ω/cm and V_0/I_0 as obtained in a current voltage plot is $1.6 \cdot 10^6$ Ω. Since in intact tissue $L \gg \lambda$, $\tanh L/\lambda = 1$. The value of $R_m = 1800\ \Omega \cdot cm^2$ is obtained from measurements in isolated surface cells where the low core resistance is absent and therefore total observed resistance $R = R_m$. Thus, λ may be calculated from Eqn. 1 and is approx. 1400 μm. The core resistance R_c is defined by:

$$R_c = \frac{a}{\lambda} \cdot \frac{V_0}{I_0} \tanh \frac{L}{\lambda} \quad (2)$$

where a is the crosscut area of the "cell cable". For surface cells, R_c is thus about $50\ \Omega \cdot cm$.

Calculations similar to those given for surface cells are more difficult in the case of oxyntic cells. Verifiable puncture of oxyntic cells in intact mucosa is at present technically not possible since localization of the electrode tip deep in the tissue has not been attempted. Thus the current voltage plot (Fig. 6) allows only comparison of isolated oxyntic cells with surface cells of intact mucosa. It may, however, not be permissible to compare properties of two different cell systems. Furthermore, the assumption that $L \gg \lambda$ cannot readily be made for Necturus gastric tubules. Gastric tubules are relatively short (about 0.5 mm) and electrical coupling between oxyntic cells and surface cells has not yet been demonstrated. An attempt was, therefore,

made to measure λ directly with a two-electrode system in isolated gastric tubules. A representative experiment is shown in Fig. 7. It is apparent that a 50 % attenuation of the PD response occurs across a few (3–5) cells. However the measurement of cell separation assumes that there is a direct path between the two electrodes, and no branching in this pathway. With these reservations, the apparent space constant in the tubule is of the order of 150 μ, since the cell diameter is 30 μ. If true, an additional artefact has to be considered, namely, rupture of the intercellular junctions by the action of pronase used in the tubule preparation.

Alternatively, the slope of the voltage current curve for the isolated tubule ($7.0 \cdot 10^6$) can be used for the calculation of λ and R_c by the same method as for the surface cell. With this method the space constant is about 400 μ, and the core resistance is about 800 $\Omega \cdot$cm. Again, due to the pronase treatment, these values are subject to revision when techniques for definitive puncture of oxyntic cells in the intact mucosa become available.

ACKNOWLEDGEMENTS

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