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Membrane and Junctional Properties of the Isolated Frog Lens Epithelium

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Summary. The isolated frog lens epithelium can be maintained intact in both appearance and electrical properties for more than 24 hours. The mean resting membrane potential was −80 mV and the cells were depolarized by both high potassium and low calcium Ringer's solution in a manner very similar to that of the whole lens. The epithelial cells were found to be well coupled using both electrical and dye-injection techniques. Electrical coupling was measured using separate current-injection and voltage-measuring electrodes and the relationship between the induced voltage and distance from the current-passing electrode could be well fitted by a Bessel Function solution to the cable equation. The values obtained from the fit for the membrane and internal resistances were 1.95 Ωm^2 and 25 Ωm, respectively. Exposure to octanol (500 μM) or low external Ca^{2+} (<1 μM) failed to disrupt significantly the intercellular flow of current. There was evidence to suggest that *raised* intracellular calcium does, however, uncouple the cells. Dye coupling was investigated by microinjecting Lucifer Yellow CH into single epithelial cells. Diffusion into surrounding cells was rapid and, in control medium, occurred in a radially symmetrical manner. In contrast to the electrical coupling data, dye transfer appeared to be blocked by exposure to 500 μM octanol and was severely restricted on perfusing with low external calcium. Differences between the electrical and dye-coupling experiments indicate either that there are two types of junction within the cell and only the larger type, permeable to Lucifer Yellow, is capable of being uncoupled or that there is only one large type of junction which can be partially closed by uncoupling agents.

Key Words lens · epithelium · membrane · dye transfer · communication · coupling

Introduction

Studies of the amphibian lens have been the major source of information concerning the electrical properties of lens membranes. Duncan (1969) first showed, using a two-internal microelectrode method, that the internal resistance of the toad lens was negligible compared with the surface membrane resistance. This electrical property has also recently been found in the mammalian lens (Kuszak et al., 1985; Lucas et al., 1987). The low internal resistance is probably due mainly to the presence of gap junctions between opposed fiber membranes (Lo & Harding, 1986). A very recent electrical study of Mathias, Rae and Riquelme (1986) indicates that these junctions can be closed, at least in the outer cortex of the frog lens, by decreasing internal pH. The junctions are similarly sensitive to increasing internal calcium (Duncan & Jacob, 1984). The whole lens electrical data mainly concern the properties of fiber-fiber cell coupling and little is known of the extent of epithelial-epithelial cell communication apart from a dye-injection study in the chick demonstrating that normally Lucifer Yellow passes readily from one epithelial cell to the next (Schuetze & Goodenough, 1982; Miller & Goodenough, 1986). It appears in the chick that dye injected into the fibers also passes to the epithelium (Miller & Goodenough, 1986) and similarly current injected into frog lens fibers can be detected in the epithelium (Rae & Kuszak, 1983). In the whole lens studies, because of the complexity of the different pathways, it is difficult to estimate for a molecule or ion entering the lens the relative importance of the epithelium-epithelium route versus fiber-epithelium.

The isolated epithelium, on the other hand, presents a two-dimensional monolayer, ideal for both dye- and current-injection studies. The relatively simple geometry involved is extremely important in the latter case as conventional cable theory can be applied to obtain estimates of both membrane and cell-cell resistance. The isolated epithelium has been used in a number of patch-clamp studies of epithelial membrane channels (Rae & Levis, 1984; Jacob, Bangham & Duncan, 1985; Cooper et al., 1987) and it has also been used to investigate the developmental biology of lens cells (Campbell & McAvoy, 1984). However, in none of these studies were there investigations of the long-term stability of the membrane characteristics of the preparation or of a comparison of the membrane properties of the isolated epithelium versus the

whole lens. More importantly, there have been no studies of cellular communication with this preparation.

Materials and Methods

Preparation

The amphibian *Rana pipiens* was used in this study. After decapitation, the eyes were removed and the lens dissected free by a posterior approach (Duncan, Patmore & Pynsent, 1981). The lens was then placed anterior face down on a Sylgard® pad at the center of a small tissue-culture dish and two fine steel needles were inserted into the lens to secure it to the pad. After an incision was made in the posterior surface, two more needles were inserted into the cut so that the capsule could be pinned flat onto the Sylgard pad. The lens fiber mass was then lifted free and the remaining fibers adhering to the periphery of the epithelium were removed with fine forceps. The preparation was mounted on the stage of an inverted microscope (Leitz Diavert) and perfused with an amphibian Ringer's solution (18°C) of similar composition to amphibian aqueous humour (mM: 105 NaCl; 2.5 KCl; 2 $CaCl_2$; 1 $MgCl_2$; 6.5 $NaHCO_3$; 5 HEPES; 2 D-glucose; adjusted to pH 7.4 with NaOH).

Electrical Recordings

Recordings were made with finely tipped glass microelectrodes pulled on a Narashigi vertical electrode puller. The glass had an external diameter of 2 mm and contained an internal capillary for ease of filling. Satisfactory electrodes for intracellular measurements when filled with 2 M KCl had resistances of 125 to 200 MΩ when measured in the Ringer's solution. Conventional high-impedance current-passing and voltage-recording circuitry was used (*see* Duncan et al., 1981) and the signals were amplified and displayed on an oscilloscope (Philips PM 322) that had been modified to output the amplified signals to an analog tape recorder (Racal 4DS) and chart recorder (Bryans Southern).

The current-passing and voltage-measuring electrodes were advanced to the surface of the epithelium. A "dimpling" of the cells could be observed under phase optics and a small deflection observed on the oscilloscope screen when the surface was reached. A gentle tap on the metal base-plate supporting the system then generally resulted in simultaneous large deflections (≈ -80 mV) on both electrode traces indicating that the electrodes had entered the epithelial cells. The current-passing electrode was connected via a current-voltage transducer set on 1 mV/nA enabling measurement of the current passed. Current pulses in the range 1 to 20 nA produced voltage deflections in the range 1 to 20 mV.

Dye Injection

Glass micropipettes without the internal filament were filled from the tip with a 30 mg/ml solution of Lucifer Yellow CH (Sigma) in injection buffer of the following composition (mM): glutamatic acid; 140 KOH; 1 $MgCl_2$; 1 dithiothreitol buffered to pH 7.2 with 40 mM citric acid (Izant, Weatherbee & McIntosh, 1983). After the cells had been microinjected in perfusing solution, they were left for approximately 15 min before the epithelium was fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline (P.B.S.) solution. The epithelium was then washed in P.B.S., detached from the Sylgard and mounted on a microscope slide. The cells were visualized with phase and epifluorescence optics (Zeiss filter set 10 for FITC fluorescence).

The pressure injection procedure does cause the cell to swell slightly (*see* Fig. 5*b*) and we calculate that a volume equal to 10% of the cell volume is injected. This type of swelling has been reported by a number of investigations but does not appear to inhibit normal cell function (Stewart, 1978). Injected cells of early stage amphibian embryos, which respond with a similar swelling, grow into normal tadpoles (Guthrie, 1984).

Results

The isolated frog epithelium, when bathed in a simple Ringer's solution, remains unchanged in appearance for more than 24 hr. The shapes of individual cells remain constant and the cell boundaries can clearly be seen (Fig. 1). The membrane potential recorded within 1 to 4 hr after dissection is -79.0 ± 0.9 mV ($n = 0.94$), and 24 to 28 hr after removal it is -68.0 ± 3.2 mV ($n = 17$). The area of epithelium readily available for electrophysiological studies was approximately 2 mm^2. In this central area the fiber cell mass could be cleanly removed from the epithelium, but this was not possible at the margins (the bow region of the lens) where cell division and elongation occurs. The fiber cells are more firmly attached in this area. The membrane potential did not appear to be a function of position within the central area. If two electrodes were placed in separate cells they recorded the same voltage to within 2 or 3 mV. However, the membrane potential was very sensitive to changes in the composition of the perfusion medium. Replacing Na^+ by K^+ led to a rapid depolarization of the membrane potential (Fig. 2*a*) and the relationship between membrane potential and external K^+ (Fig. 2*c*) was very similar to that obtained from the whole frog lens (Patmore & Duncan, 1980). The membrane potential is also rapidly depolarized by reducing the external Ca^{2+} concentration and if the exposure is brief, the depolarization is reversible (Fig. 2*b*) just as it is in the whole frog lens (Delamere & Paterson, 1978). Prolonged exposure (>30 min) leads to marked cell swelling (*cf.* Fig. 5*f*). As a result the cells could easily be pulled free from the underlying capsule by, for example, withdrawing the intracellular electrode.

Resistance-measurement experiments did reveal significant differences between the behavior of the frog epithelium and whole lens. In the whole lens the current-voltage curve shows marked outward rectifying properties (Patmore & Duncan, 1980) while the isolated epithelium acts as weak inward rectifier (Fig. 3). In the epithelium it is not

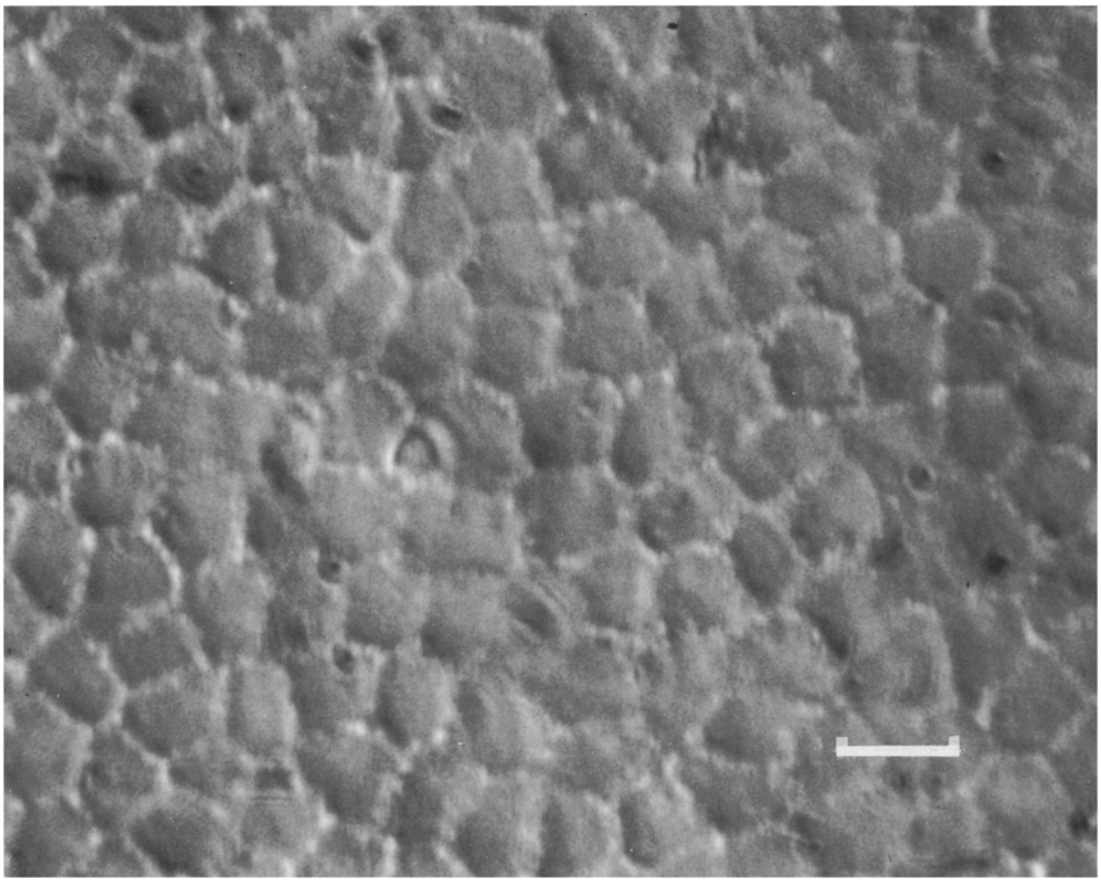

Fig. 1. Isolated epithelium of the perfused frog lens. Scale bar = 16 μm

possible to say at present whether the rectifying properties reside in the membranes or in the intercellular resistance. In the whole frog lens the measured resistance is relatively insensitive to the positions of the current-passing and voltage-measuring electrodes (Duncan, 1969, 1973; Eisenberg & Rae, 1976). In the isolated epithelium, the voltage response induced in one cell to a step of current in another is very sensitive to the separation of the electrodes (Fig. 4). When the electrodes are close together the measured resistance is of the order of 1 to 2 MΩ, which compared with a value of 5 to 10 kΩ for the whole lens (Duncan, 1969; Patmore & Duncan, 1980). The resistance falls sharply with distance, presumably because only a proportion of the current entering a cell is passed on to the neighboring cells. Some of the current leaves through the surface membrane. In this respect, the epithelium is acting as a two-dimensional cell syncitium (Loewenstein, Nakas & Socolar, 1967) and the simple geometry of the isolated epithelium permits a mathematical modelling of the relationship between the steady-state magnitude of the induced voltage and the separation of the current and voltage electrodes (Jack, Noble & Tsien, 1975).

In order to be able to apply cable theory equations to interpret the spread of current from the current-passing electrode, it is essential that other possible routes, such as adhering fiber cells, are absent. The few fiber cells that remained on the apical surface could in fact be easily removed and Campbell and McAvoy (1984) have direct evidence from fluorescent antibody studies that no fiber-specific crystallines remain in the vicinity of the central region of the isolated rat epithelium. We have carried out a similar study of isolated bovine epithelium with fluorescent antibodies directed against bovine MP26. Again no fluorescence could be detected in the central regions, although a positive signal was obtained from fiber cells remaining on infoldings of the peripheral capsule. We are confident, therefore, that in the frog, and indeed all the species we have studied (human, rat and bovine) the central 2-mm^2 area of epithelium is free from adhering fibers. This preparation is therefore one in which current flow is restricted to one type of cell.

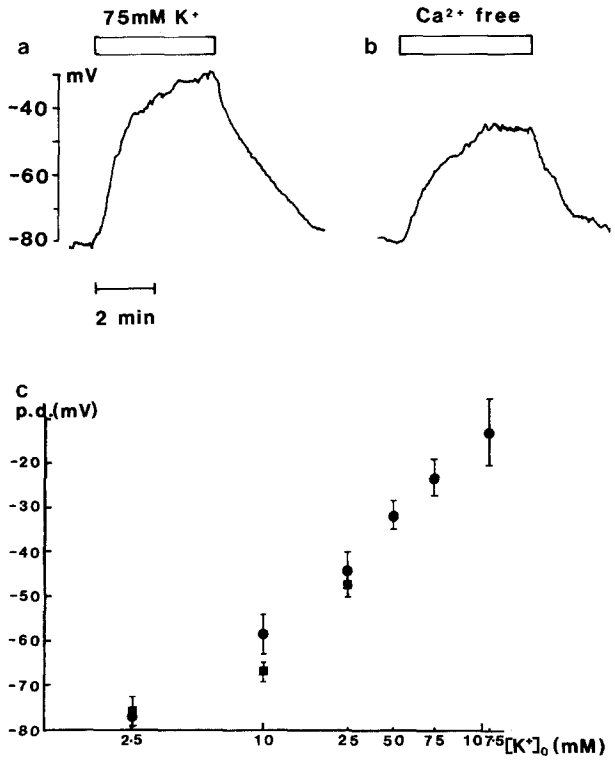

Fig. 2. Effect of changing external K^+ and Ca^{2+} on the membrane potential of continuously perfused frog epithelial cells. (*a*) Increasing K^+ leads to a rapid depolarization of the membrane potential and recovery is complete 5 min after restoring the control solution (2.5 mM K^+). (*b*) The preparation was perfused with Ca^{2+}-free solution 10 min after the end of the high-potassium experiment. 1 mM EGTA was added to reduce the external calcium activity to below 1 μM. (*c*) Relationship between membrane potential and external potassium concentration in the perfused epithelium (●). The data are expressed as the mean ± SE of at least four measurements in each case. The whole lens data (■) are taken from Patmore and Duncan (1980)

If it is assumed that the current is injected from a point source and spreads in a radially symmetrical manner outwards, then the current density will fall off with distance partly due to the fact that it is spreading into a larger and larger volume, but also due to the fact that it will pass out from the cell across the apical and basal surface membranes. If we assume that the specific resistance (R_m) of both sets of membranes is the same, then the relationship between the steady-state voltage ($\bar{V}_{o,j}$) measured at a cell n_j cell diameters from the current-passing electrode is given by:

$$\bar{V}_{o,j} = I_o \frac{R_i}{2\pi b} K_0 \left[\frac{n_j}{\lambda}\right] \quad (1)$$

where K_0 is a zero-order Bessel function (Watson, 1962), R_i is the radial resistance (Ωm), b is the distance between the apical and basal membranes (8 μm) and $\lambda = \sqrt{(R_m b/2R_i)}$. The data are more conveniently expressed in terms of resistance ($R_{o,j}$) where $R_{o,j} = \bar{V}_{o,j}/I_o$ and the points obtained (Fig. 4*b*) could be well fitted by Eq. (1). The values obtained for R_i and R_m were 25 Ωm and 1.95 Ωm^2, respectively.

The space constant (λ) is greater than 20 cell diameters and so the epithelial cells can be considered to be well coupled electrically. Expressed in another way, the resistance falls to half the single-cell resistance after about 3 cell diameters or 50 μm. This compares with distances of 100 μm in salivary gland epithelia (Loewenstein & Kanno, 1965) and ciliary epithelia (Green et al., 1985). The differences in characteristics of the current spread are probably due partly to the fact that the cells are larger in

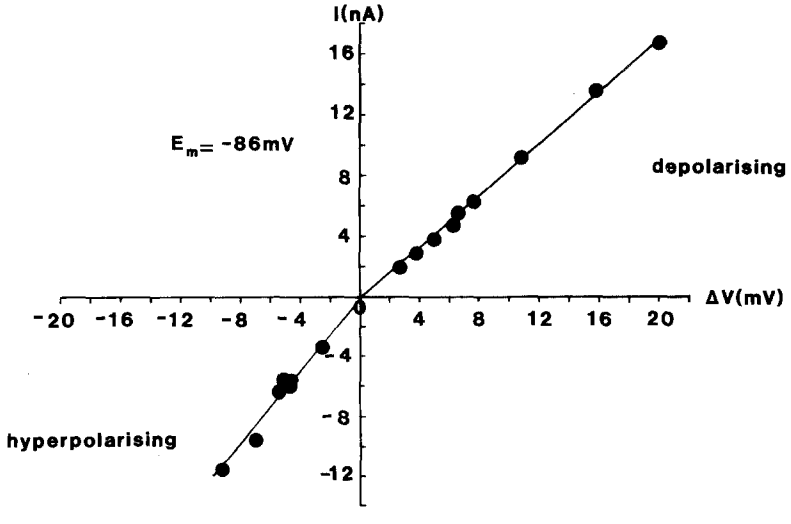

Fig. 3. Current-voltage characteristics of perfused frog epithelial cells. The voltage-recording electrode was placed two cell diameters (approx. 30 μm) from the current-injection electrode. The same resting membrane potential (−86 mV) was recorded on both electrodes. The resistance was approximately 1.3 MΩ in the depolarizing direction and 0.83 MΩ with hyperpolarizing currents, so the cells have weak inward rectifying properties

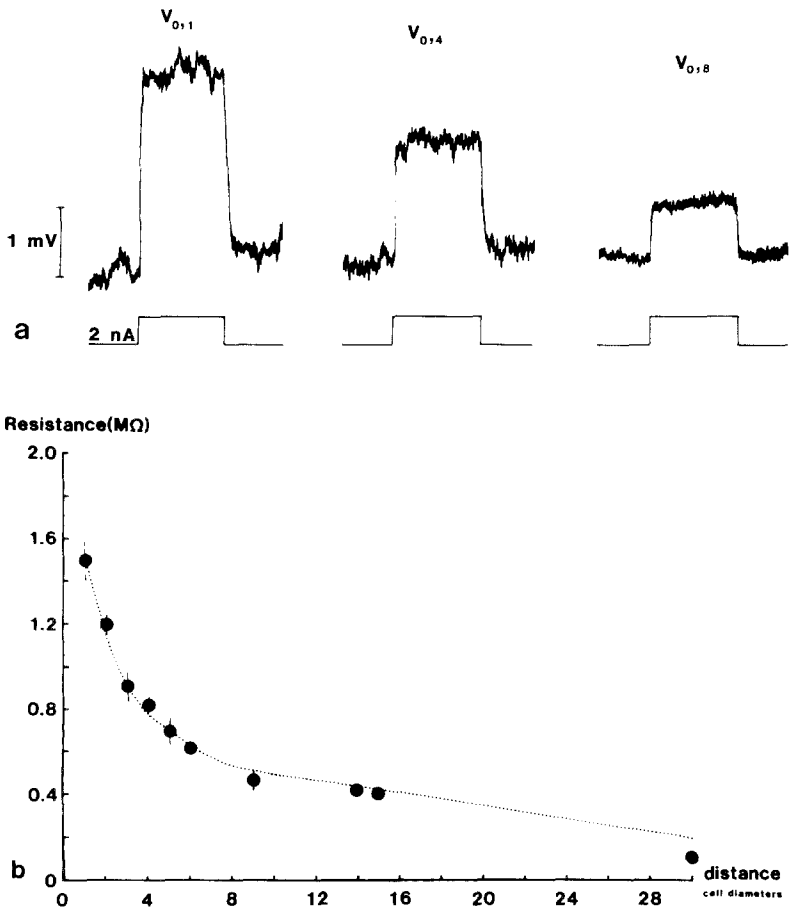

Fig. 4. (*a*) Voltage-response of the lens to a square pulse of current. The current electrode was positioned at the center of the epithelium (I_0) and the distance of the voltage-recording electrode was measured relative to the current electrode in terms of cell diameters apart. $V_{0,1}$, for example, denotes that the voltage electrode was placed 1 cell diameter from the centrally positioned current electrode. (*b*) Resistance of the epithelium as a function of electrode separation. The dashed line gives the best fit of Eq. (1) to the data. The resistance values obtained with two electrodes in the same cell ($V_{0,0}$) are not given as the impalement was usually very short-lived (<5 min) and the resistance increased with time (*see* Fig. 6)

these preparations but also because current is more constrained to flow along the long axes of these tissues rather than along a plane as in the lens epithelium.

The two-dimensional communicating properties of the frog epithelium can be clearly seen in dye-injection experiments. Lucifer Yellow injected into one cell in control solution rapidly spreads out into surrounding cells in a radially symmetrical manner (Fig. 5). On some occasions (approximately 10% of injections), however, the dye remained in the injected cell and is presumably due to injection damage causing closure of dye-communicating junctions. We also found during electrical measurements that individual cells could become electrically uncoupled from the surrounding cells. In this case the refractive properties of the cell changed, the membrane potential declined towards zero, and injection of current at a distant electrode did not produce a voltage response in this type of cell. However, when the two electrodes were in the same cell, the voltage response to a current pulse increased when the membrane potential began to decline with time (Fig. 6). This type of cellular uncoupling, due to injury of the cell, is probably due to a flow of calcium into the cell as electrical uncoupling did not occur when calcium was absent from the medium (Fig. 6).

Interestingly, differences between electrical- and dye-uncoupling measurements emerged where a number of uncoupling agents were applied to the epithelium. In the presence of octanol, which uncouples a range of epithelial cell types from rat hepatocytes (Spray et al., 1986) to *Obelia* photocyte effector cells (Dunlap, Takeda & Brehm, 1987), there was little effect either on the single-cell resistance (Table) or on the coupling ratio for electrodes separated by several cell diameters (Table). For example the measured resistance three cell diameters apart was 1.7 MΩ in 500 μM octanol, which compares with 0.9 MΩ in control. However, the spread of dye from cell to cell appeared to be totally blocked (Fig. 5*c*). The appearance of the cells also changed when viewed through phase optics and the borders between the cells became much less pronounced (Fig. 5*d*).

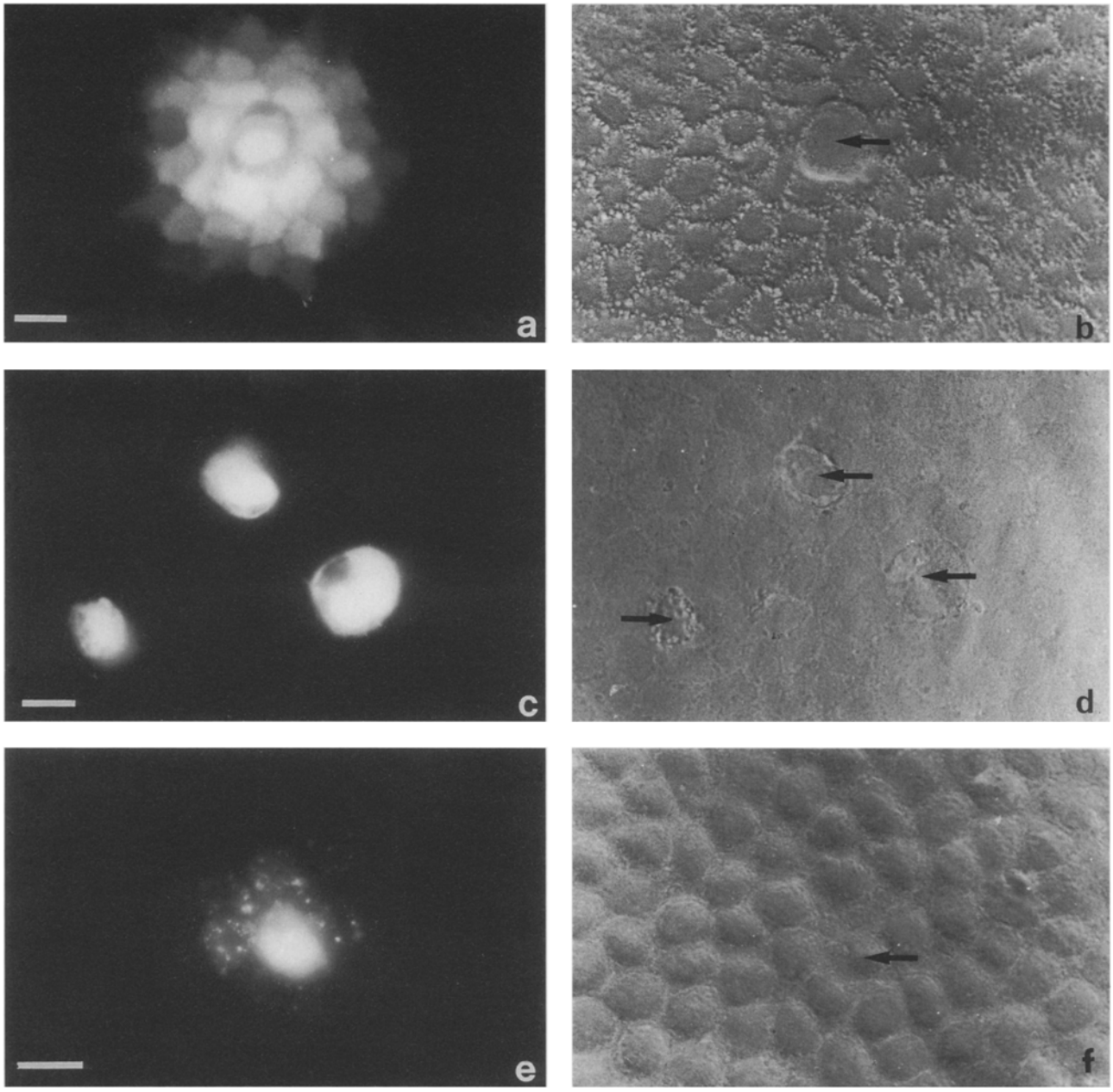

Fig. 5. Epifluorescence (*a*, *c*, *e*) and phase-contrast (*b*, *d*, *f*) views of the isolated frog epithelium. (*a*) Spread of Lucifer Yellow from a single injected frog epithelial cell (arrowed in *b*). In control perfusing solution the dye can clearly be seen to have diffused to at least 50 other cells in a radially symmetrical manner. (*b*) Phase-contrast image of the same group of cells. (*c*) Three cells injected in control perfusing solution with 500 μM octanol added show no spread of dye (within the limits of sensitivity of this system) when injected with Lucifer Yellow. (*d*) Cell borders can be seen to be less distinct, presumably because of the surfactant effect of the long-chain alcohol. (*e*) A single frog epithelial cell, perfused with calcium-free Ringer's solution for 30 min and then injected with Lucifer Yellow. There is an abnormal spread of dye (*cf. a*) and the cells are also swollen (*f*). The calibration bar in each case corresponds to 20 μm

Exposure to calcium-free media electrically uncouples salivary glands (for example Loewenstein et al., 1967). However, on exposure of lens cells to low calcium, there was no increase in single-cell resistance (Fig. 6) nor a decrease in the current passed to adjacent cells (Table), although the membrane potential depolarized (Fig. 2*b*) and there was pronounced cell swelling (Fig. 5*f*). Diffusion of Lucifer Yellow from cell to cell was, however, greatly reduced (Fig. 5*e*) and the dye appeared to adhere to

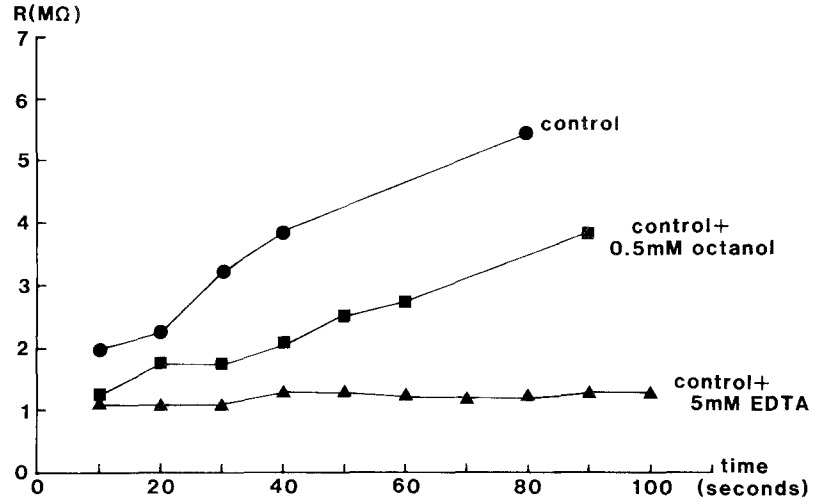

Fig. 6. Resistance of the frog epithelium measured with two electrodes in the same cell. In this case the measurements were not as stable as those obtained with the electrodes in different cells (Figs. 2 and 3) and invariably, soon after impalement, the membrane potential recorded on both electrodes fell. When one of the electrodes was used to pass current, the resistance was found to increase. A decrease in resistance would have been expected if the electrodes were simply coming out of the cell and so this increase implies cell uncoupling. The increase did not, however, occur in calcium-free media

Table.

Solution	$R_{0,0}$	$R_{0,1}$	$R_{0,3}$	E (mV)
Control	2.6 ± 0.3	1.55 ± 0.04	0.9 ± 0.1	−78.8 ± 0.9
	(n = 18)	(n = 50)	(n = 4)	(n = 94)
Octanol	2.4 ± 0.1		1.7 ± 0.01	−74.3 ± 2.4
500 μM	(n = 3)		(n = 11)	(n = 17)
Ca-free	1.3 ± 0.03	1.4 ± 0.1		−27.9 ± 3.6
+ 1 mM EDTA	(n = 9)	(n = 9)		(n = 13)

The resistance (MΩ) was measured by placing the current-passing electrode at the center of the epithelium and recording the voltage deflection in the same cell ($R_{0,0}$), a neighboring cell ($R_{0,1}$), or in a cell three cell diameters distant ($R_{0,3}$). The data are given as the mean ± SE

discrete elements within the immediately surrounding cells. Cell swelling and dye uncoupling were totally reversed after overnight incubation in control medium and there was also recovery of the membrane potential to within 5 mV of the control value.

Discussion

The isolated frog lens epithelium is a very robust preparation as it can be maintained in vitro in a simple artificial aqueous humour solution with little loss of membrane potential. It is therefore ideal for an investigation of the fundamental membrane properties of lens epithelial cells. The control membrane potentials reported here are much higher than previously reported values of about −60 mV obtained during patch-clamp studies (Jacob, 1984; Rae, 1985) but the difference cannot be one simply of technique as recent patch-clamp studies carried out on the same group of frogs used in the present study (Patmore and Duncan, *unpublished data* obtained with a List patch-clamp system) yielded membrane potentials in the range −80 to −90 mV. We have found in fact that in order to maintain high lens membrane potentials in *Rana pipiens* the frogs have to be kept at an ambient temperature of >12°C. This is especially important during the winter months when we have consistently recorded stable voltages of the order of −60 mV from specimens kept at below 10°C (Williams & Duncan, *unpublished data*). The membrane potentials are also much higher than those reported by Rae and Kuszak (1983) in epithelial cells in the intact frog lens, but in that study, an enzymatic pretreatment was necessary which apparently caused a 10-mV depolarization in membrane potential of the whole lens. The present values are, however, of the same order as those previously reported for whole frog lenses by Patmore and Duncan (1980) and Delamere, Duncan and Paterson (1980). It is likely, therefore, that removing the anterior epithelium from the underlying fiber mass causes little damage to the epithelial

cells. As the epithelium does appear to be electrically (Rae & Kuszak, 1983) and dye (Miller & Goodenough, 1986) coupled to the fibers this indicates that the coupling is extremely labile. This would of course be an advantage to the lens where new fiber cells are continually growing over older underlying fibers. The very high resting potential of the frog epithelium indicates that the membrane characteristics are dominated by potassium channels. The very great sensitivity of the potential to changes in external potassium found in the present study (Fig. 2) supports this idea.

The depolarization of the membrane potential in the presence of low calcium is probably due to the activation of nonspecific cation channels in the apical surface of the epithelial cells and patch-clamp studies have shown that both the lifetime of the channels and their sodium-potassium selectivity changed on lowering external calcium (Jacob et al., 1985; Cooper et al., 1986). The cells would also be expected to swell with a net entry of sodium and there is evidence that this did indeed occur (Fig. 5*f*). The fact that the changes in cell shape and depolarization of membrane potential were reversible after overnight incubation in control medium shows that sodium extrusion mechanism is still active and also that the changes in channel activity can be reversed.

The values for the membrane resistance (R_m) and intercellular resistance (R_i) obtained by applying current diffusion theory to the electrical impedance data in Fig. 4(*b*) can be compared directly with estimates obtained from other techniques. Rae (1985) applied patch-clamp techniques to single, enzymatically dissected frog epithelial cells and obtained a value of 10^9 Ω (computed from Fig. 4 of Rae, 1985) for the input resistance. Assuming his cell had the same dimensions as our central epithelial cells (13 × 13 × 8 μm), then the specific resistance of the cell membrane can be computed to be approximately 1.4 Ωm^2, which is close to the value of 1.94 Ωm^2 that we obtain from the present data. The intercellular resistance we calculate (25 Ωm) is very much higher than that of simple Ringer's solution (1 Ωm) and suggests that the junctions, although lowering the cell-cell resistance considerably, still impede to some extent the flow of ions. Eisenberg and Mathias and their collegues have applied spherical diffusion theory to impedance data obtained from whole lenses and their estimates of the intercellular resistance of amphibian lenses range from 6.25 Ωm in their earlier reports (Mathias, Rae & Eisenberg, 1979) to 90 Ωm in a more recent publication (Mathias & Rae, 1985). Mathias, Rae and Eisenberg (1981) point out that the whole lens is a nonuniform spherical syncitium, and have suggested that the lower values for R_i correspond to longitudinal or cytoplasmic resistance, while the higher values relate to radial or interfiber resistance. If this is indeed the case then the cell-cell resistance in the epithelium is significantly lower than the fiber-fiber resistance in the whole lens.

As the whole frog lens represents a relatively well-coupled syncitial system, it is possible to compare the specific resistance of the whole lens fiber membrane ensemble (units Ωm^2) with that of a single epithelial cell (1.94 Ωm^2). The resistance of a *Rana pipiens* lens is approximately 10 kΩ, while the geometrical surface area is of the order of 0.25 × 10^{-4} m^2 (Duncan et al., 1981). The specific resistance of 0.25 Ωm^2 is thus lower than the specific resistance of the epithelial cell membrane. However, fiber membranes other than those at the surface would be expected to contribute to the total lens resistance and so the area multiplication factor is likely to be much higher than the simple geometrical area. The fact that the time constant of the whole lens voltage response to a step of current (≈1 sec) is much longer than the epithelial cell response (Fig. 4*a*) indicates that a very large resistance must contribute to the shaping of the whole lens response. This resistance could arise partly from the inner fiber membranes and partly from the narrow extracellular spaces between the fibers (Mathias et al., 1979). However, Duncan et al. (1981) have computed, on the basis of an equivalent circuit model, that the resistance of the superficial fiber membranes is of the order of 70 Ωm^2. This relatively high membrane resistance would greatly lengthen the response time of the whole lens ensemble.

The intercellular current pathway in the amphibian epithelium is resistant to octanol and low calcium treatments, both of which have been shown to block ionic coupling in other tissues (Loewenstein et al., 1967; Spray et al., 1986). Both of these agents, however, severely restricted the flow of Lucifer Yellow from cell to cell in the lens epithelium. It is interesting that Schuetze and Goodenough (1982) have previously reported a differential sensitivity of lens cell junctions to CO_2 treatment. They showed that electronic coupling was insensitive to CO_2 but that dye transfer could be blocked at least up to development stage 14. This suggests that, as the chick lens fiber cell develops, the communicating junctions become irreversibly locked in the open state. This does not seem to be the case in the mature frog lens (Mathias et al., 1986; Croghan, Duncan & Emptage, 1987) or in human tissue-cultured lens cells (Stewart, Marcantonio & Duncan, 1988), however, where the internal resistance (R_i) is dramatically increased on perfusing with 100% CO_2.

Rose, Simpson and Loewenstein (1977) have shown that injecting calcium into salivary epithelial cells produced a graded closing of intercellular channels so it is possible that connecting channels may exist in a number of open states. Although the present experiments do not directly show that calcium closes junctional channels in the frog epithelium, the failure of damaged cells to close in the absence of calcium (Fig. 6) suggests that it has a role to play.

Cellular communication in the lens therefore shows great diversity of behavior since not only are there differences between electronic coupling and dye transfer between cells of the same type, but epithelial-epithelial, epithelial-fiber and fiber-fiber communication have their own characteristics which may vary not only from species to species, but also at different ages within the same species. Much detailed study will be required to unfold the underlying molecular mechanisms to explain this diversity, but some preliminary work has been carried out in this area. Lo and Harding (1986) have carried out a comprehensive study of the structure and distribution of gap junctions in lens epithelium and fiber cells from a number of species and concluded that "structural diversity of gap junctions, based on connexon (sic) arrangements, was evident in lens epithelia among the species studied." For example, gap junctions with a random array of connections were found predominantly in the frog lens epithelium, while crystalline and striated configurations were mainly obtained in human and rat epithelia. Since there appear, invariably, to be more than one type of junction on any one cell the differences in sensitivity of electronic coupling and dye transfer to blockade by a number of agents (Figs. 5(*c*) and (*e*) and Schuetze and Goodenough, 1982) can be readily explained if two possible routes are available. One type of connection arrangement could provide large, very sensitive channels through which dye could pass, and another type could provide small, relatively insensitive channels through which only small ions could pass. Alternatively, of course, there could only be one basic type of channel which could exist in one or more conducting states. The higher conducting states would allow both Lucifer Yellow (mol wt 457.2) and small ions to pass while the lowest conductance state would only permit movement of the latter. Preliminary experiments on tissue-cultured human lens epithelial cells (Stewart et al., 1987) have shown that dye transfer and electronic coupling is blocked by CO_2 and in that case the lowest conducting state would be zero.

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