


Resting membrane potentials, electrical resistance, and coupling of chick retinal pigment epithelial cells in tissue culture have been measured with micropipette electrodes. Topical application of NaCl, KCl, MgCl₂, and CaCl₂ produces rapid, reversible changes in the membrane potentials of these cells. CaCl₂ uniquely induces a slow hyperpolarizing wave that can lead to barrages of depolarizing action potentials or spikes and a slow, reversible vacuolization of these cells.

Chick retinal pigment epithelium can be maintained and cloned in tissue culture, providing a potentially useful method of examining the functional properties of these cells under high power microscopic observation and isolated from other elements in the eye. During experiments designed to examine some basic physiologic properties of single cells in these cultures, we noticed an unusual phenomenon produced by Ca²⁺ which we would like to describe.

Single pigment epithelial cells, growing in monolayered colonies (Fig. 1), were penetrated with micropipette electrodes under direct vision using an inverted microscope. The cells were growing in approximately 4 ml. of culture media in Falconer plates, 5 cm. in diameter. The techniques used to culture these cells have been described elsewhere. The methodology used to record from and stimulate single cells electrically under these conditions resemble those of Nelson, Ruffner, and Nirenberg in their studies of neuroblastoma cells.

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Results are based on well differentiated cells growing in the center of mature colonies (Fig. 1); such cells are taller and relatively easier to penetrate than those, growing at the edge of a colony, which are flatter and less differentiated. All cells, cleanly penetrated, had large stable, negative membrane potentials (64 ± 13 mv; n = 285). The electrical resistance of these cells measured by passing current through the recording microelectrode and cell in a bridge circuit² was relatively low (35 ± 10 x 10⁴ ohms; n = 25). Part of this low resistance is due to electrical coupling between cells which was demonstrated in two pairs of cells using two separate micro-electrodes. Electrical coupling between pigment epithelial cells is characteristic of mature, intact retina and its existence in tissue culture adds further evidence to support the highly differentiated state of these cells.

The application of small amounts (5 to 10 μl) of 0.5 M solutions of NaCl, KCl, MgCl₂, or CaCl₂ just above the surface of the culture media and near the cells in question produced rapid, reversible changes in the membrane potentials of these cells (Fig. 2). NaCl, KCl, and MgCl₂ depolarized the cells, the K⁺ salt being more effective than the others (see Table 1). CaCl₂ produced an initial depolarization which was followed by a larger hyperpolarizing wave (Fig. 2). Half of the time this hyperpolarization was followed by a barrage of depolarizing action potentials (Fig. 2, inset). These impulses or spikes were rhythmic, all-or-none responses that usually occurred in clusters, sometimes singly but never spontaneously or after the other solutions. Fig. 3 shows an oscillograph of such a single pigment epithelial cell spike.

In addition to this electrical change, a curious, slow, transient vacuolization within these cells was also noticed after application of CaCl₂. This optical change was subtle for it was missed in all the initial experiments. Even after we suspected its presence, it only became obvious after looking at time lapse photographs (Fig. 1). The vacuolization begins at about the time the spikes appear but far outlasts them. It is not yet clear how dependent the vacuoles are on the spike discharge.

The concentration of Ca²⁺ necessary to trigger these events is not easy to measure. The quantity of solution we have used raises the overall con-
"Fig. 1. Photographs of the center of a colony of chick retinal pigment epithelium growing in tissue culture: 0, before and 20, 80, and 120 seconds after application of CaCl². Calibration at upper right indicates 25 microns.

Fig. 2. Intracellular responses of chick retinal pigment epithelial cells to local application of CaCl², NaCl, MgCl₂, and KCl. The upper two traces show the sudden negative shift of the (DC) direct current potential occurring when the cell membrane has been penetrated. The arrow indicates the time at which a test solution has been pipetted on to the surface of the culture media. The spikes induced by CaCl² are shown at a faster time base in the insets. The calibration at lower right indicates 15 millivolts vertically and 3 seconds horizontally for the main traces; 0.6 seconds for the insets. Positively is upward."
centration of Ca** in the culture media less than 0.01 millimoles. Since the solution has to be applied near the cells in question, the local concentration required to induce these responses must be higher than this. An order of magnitude estimate of this triggering concentration can be obtained by assuming that the pigment epithelium cell membrane approximates a K+ electrode, an assumption supported by the fact that K+ is very effective in depolarizing these cells (Table I). The depolarization produced by 10 microliters of 0.5 M KCl was 36±12 mv., which would be produced by about a 20 to 40 millimole increase in K+ outside the cell. The triggering concentration of Ca** is probably near this range.

It would be interesting to know whether this curious phenomenon induced by Ca** plays any role in the normal function of retinal pigment epithelium. As far as we know spikes have only been found in nerve and muscle and never in any epithelial cell.

The hyperpolarizing wave that precedes the spikes bears some resemblance to the c-wave of the electroretinogram which is a hyperpolarizing potential induced in intact retinal pigment epithelium by light impinging on the photoreceptors. Steinberg and Miller have suggested that the light-induced Na+ conductance change in the photoreceptors leads to a local reduction of extracellular K+ and hence to a hyperpolarization of the retinal pigment epithelium. Some direct support for this hypothesis has recently been obtained. If Ca** were released from the photoreceptors by light, as has also been suggested, the late hyperpolarization it produces on pigment epithelial cells could also contribute to the c-wave of the electroretinogram.

We would like to thank Drs. Fernando de Melo and Dr. Marshall Nirenberg for their assistance in this project.

References
6. Oakley, B., and Green, D. G.: The ionic basis of the c-wave of the electroretinogram, ARVO meeting abstracts, 1975, p. 5.

Survival of some photoreceptor cells in albino rats following long-term exposure to continuous light. MATTHEW M. LAVAL.

Fischer albino rats, seven weeks of age, were exposed to continuous light at 65 foot-candle incident illuminance for up to 264 days. Other Fischer rats, seven months of age, were exposed to continuous light at 140 foot-candle incident illuminance for up to 147 days. In all cases, a small percentage of the photoreceptors survived. The identification of the surviving cells as photoreceptors was made by light microscopy on the basis of nuclear heterochromatin pattern and staining and by electron microscopy by the presence of ribbon synapses and ciliary basal bodies with ciliary filaments. No outer segment membranes were observed. The percentage of cones progressively increased from the normal 1.5 per cent to about 60 per cent with increasing exposure time, indicating that cone cells are more resistant than rods to destruction by constant light.

Continuous illumination causes photoreceptor cell degeneration in normal albino rats. The rate