ELECTROCHROMIC DYES FOR VISUALIZATION AND ASSESSMENT OF INTRACELLULAR POTENTIAL IN THE GASTRIC MUCOSA OF RAJA ERINACEA

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Electrochromic dyes have been used on single cell preparations (Sims et al., Biochemistry 13:3315, 1974) but less successfully on epithelial tissues (Leader and Macknight, Fed. Proc. 41:54, 1982). We report that an electrochromic dye allows visualization and differentiation between cells of different potentials. Figure 1 shows the chamber system used, which permits visualization without free surface distortion while maintaining sufficient fluid flow for normal tissue function. The chamber is mounted on a Nikon Labophot microscope, and can be illuminated from below with white light and from above (epi-illumination) with filtered light from a halogen lamp for excitation of dye fluorescence. A Leitz 32X long working distance objective allows focusing the image to the eyepieces, to a 35 mm camera, or to a Hitachi Newvicon video camera.

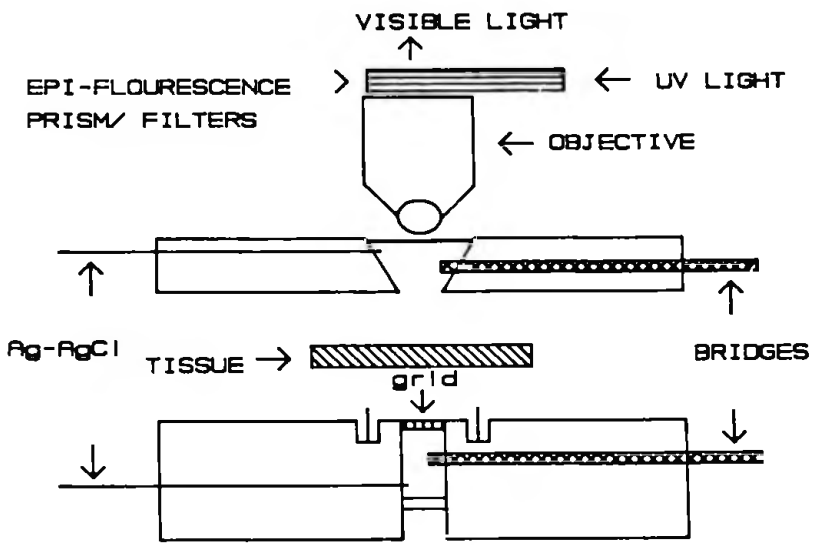
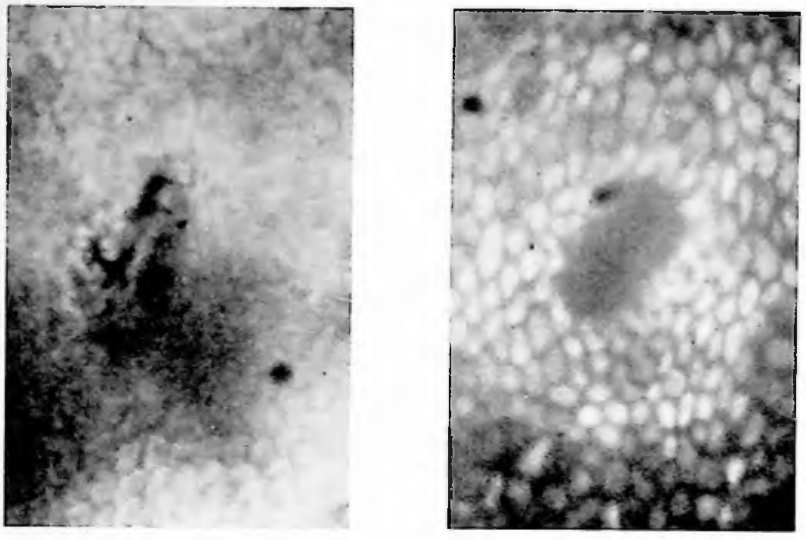


Figure 1. Diagram of the chamber system.

The gastric mucosa of the little skate was prepared as previously described (Kidder & Kidder, Bull. MDIBL 22:30, 1982), using buffered elasmobranch Ringer's on both surfaces, and mounted in the chamber with the mucosal surface upwards. The electrochromic dye (Di-O-C5(3), Molecular Probes) was dissolved in the mucosal bathing solution at 10^{-6} M, a concentration which has no detectable effect on transport in frog skin or gastric mucosa.

Figure 2a is a micrograph of the tissue transilluminated by white light. The image is severely degraded by the distortion of the illuminating beam by passage through the tissue. Figure 2b is this same tissue with the dye excited with blue light, with the fluorescence signal observed through a minus-blue filter. Two cell types can be distinguished on the basis of their fluorescence intensity. Most of the surface is covered by surface epithelial cells which show an intense fluorescence. The secretory pits have a dark (acellular) center, and are bordered by cells which are more fluorescent than

the center of the pit. Individual surface cells are clearly visible, and in favorable cases boundaries between the cells bordering the pit can be seen.



(a) (b)

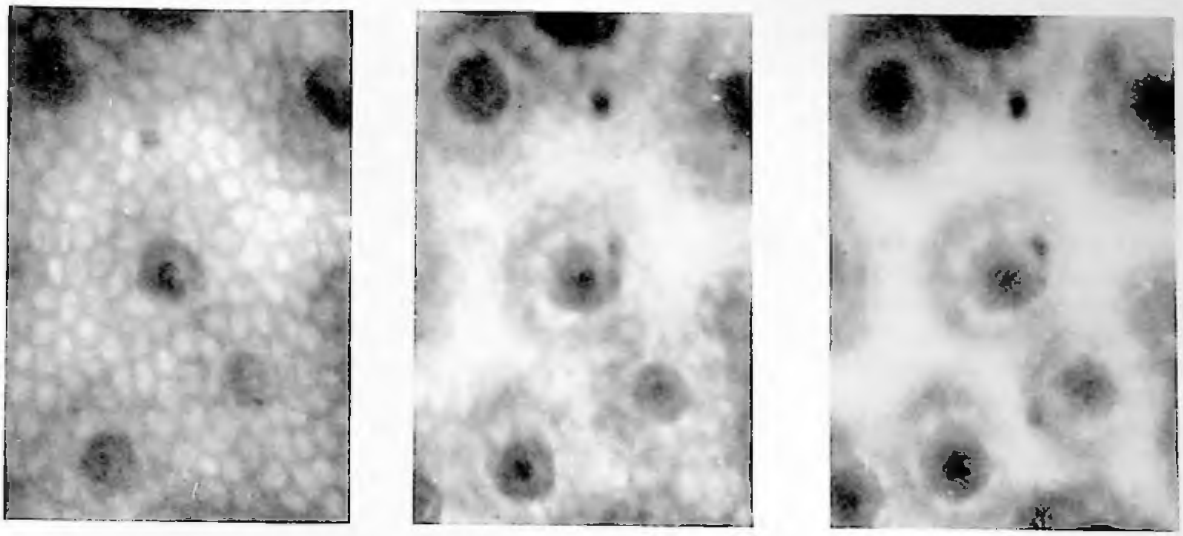
Figure 2. Visible light (a) and fluorescence (b) micrographs of a typical tissue, showing the distinction between surface and pit border cells. The original (color) image has greater visual contrast, lost in these prints. Note the differences in fluorescent brightness between the surface cells, the cells bordering the gastric pits, and the pit lumen.

Scale 20μ

Figure 3 shows a series of optical sections at progressively deeper levels. As the focal plane is moved below the surface, the fluorescence from the surface cells fades. The area occupied by the oxyntic cells apparently expands with depth without showing brightness changes. The pit lumen also expands with depth and becomes less distinct as the bottom of the pit is approached. Of interest is the observation (Figure 3d) of a ring of fluorescence which delimits the gastric gland.

Since the dye has no effect on acid secretion or the electrical parameters (unpublished observations) it can be used as a "vital stain" in this chamber to visualize the surface of an epithelial tissue with considerable clarity, which should permit placement of microelectrodes, etc., with confidence. However, the response of the dye to changes in transmembrane potential may give valuable additional information.

Leader & Macknight maintained that since the dye carries a positive charge, it would be preferentially accumulated by hyperpolarized cells. They then postulated that the dye is rapidly destroyed by intracellular enzymes, so these cells do not fluoresce but have a higher transmembrane dye flux then depolarized cells. This lowers the dye concentration in the adjacent unstirred layers, giving these cells a lower fluorescence signal. This model predicts that the highest fluorescence would be observed in the bulk solution, since this would be the region of maximum dye concentration. However, we are observing the tissue through 1 mm of dye-containing solution, through which the excitation beam is passing. Since the solution is 100 times thicker than the cells, the Leader & Macknight model predicts that 99% of the fluorescence should come from the bulk solution, obscuring any tissue image. Our clear



(a) (b) (c)

(d)

Figure 3. Fluorescence micrographs of the tissue shown in Figure 2, with the microscope focused (a) 10, (b) 20, (c) 30 and (d) 40 microns below the mucosal surface. Note the fluorescent "ring" around the gastric gland at depth.

image shows that the fluorescence is emanating from the tissue cells.

The cells bordering the pits are equally fluorescent at any depth, suggesting electrical coupling between the deep (presumably oxyntic) cells and those toward the mucosal surface (mucous neck cells or their equivalent). Such coupling between unlike cell types is not thought to be common.

It is clear that this technique can produce clear visualization of the cells of this tissue, can distinguish between classes of cells (apparently on the basis of their intracellular potentials) and can be performed without altering the physiology of the tissue. It should thus prove of great utility in studies of transporting epithelia. (Supported by NIH AM-35611 to GWK)