RHODAMINE 123 AS A PROBE FOR DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

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The highly active secretory processes sustained by the elasmobranch rectal gland are possible because of the abundance of mitochondria contained in these epithelial cells. Mitochondrial biogenesis and translocation of proteins into these mitochondria appears to be substantial due to the high density of these organelles and thus the rectal gland may provide a very active model for the study of mitochondrial import. The initial characterization of such mitochondria may be approached using the dye, rhodamine 123. Cellular uptake of rhodamine 123 is thought to be dependent only on membrane potentials, since it is considered to be freely diffusible across biomembranes, and distribution into cells is described as Nernstian. This lipophillic cation will therefore be accumulated in viable cells and concentrated in actively respiring mitochondria due to their higher membrane potential. Rhodamine 123 has the added advantage of low toxicity (Johnson, L.V., Walsh, M.L. and Chen, L.B. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 990-994).

Incubation of 3 mm thick sections of dogfish (Squalus acanthias) or little skate (Raja erinacea) rectal gland with 10 ug/ml rhodamine 123 in elasmobranch saline or cell culture medium for 10 minutes was followed by three washes of solution not containing the dye. The sections were then photographed using an inverted epifluorescence microscope and a filter set for fluorescein isothiocyanate (near optimal for rhodamine 123). Figure 1 a,b,c demonstrates that intact dogfish tubules and 1d, intact skate tubules may be visualized and at the higher magnifications the density of mitochondria is such that the detailed morphology of the cell can be seen. A high degree of interdigitation of the cells is obvious as is the convoluted cell boundary.

Staining of dogfish rectal gland cells grown on glass coverslips or a plastic surface indicates that the majority of cells in a confluent layer do not take up the dye and those that do lie at the periphery of the cell mass (Figure 2a, rhodamine 123 staining and 2b, phase contrast of the same confluent cell layer). Cells grown on a permeable substrate, as in a Millicell chamber, accumulated the dye only when it was applied from the basolateral side (Figure 2c, basolateral application; 2d, apical application). Preincubation of the cells with mitochondrial uncouplers such as dinitrophenol or FCCP prevented the punctate accumulation of the dye. Application of these inhibitors after rhodamine 123 uptake resulted in the dissipation of the mitochondrial fluorescence. These results suggest that the uptake of rhodamine 123 is polarized in these cells and that all biomembranes are not equally permeable to this lipophillic dye as has been assumed.

The morphology and number of mitochondria is difficult to discern in these cells due to the large number and close packing. High magnification of well spread, single cells shows a granular lacework of mitochondria due to the extensive plasma membrane infolding.

This work demonstrates that rhodamine 123 is suitable for the study of elasmobranch mitochondria and may be used on intact and non-transparent

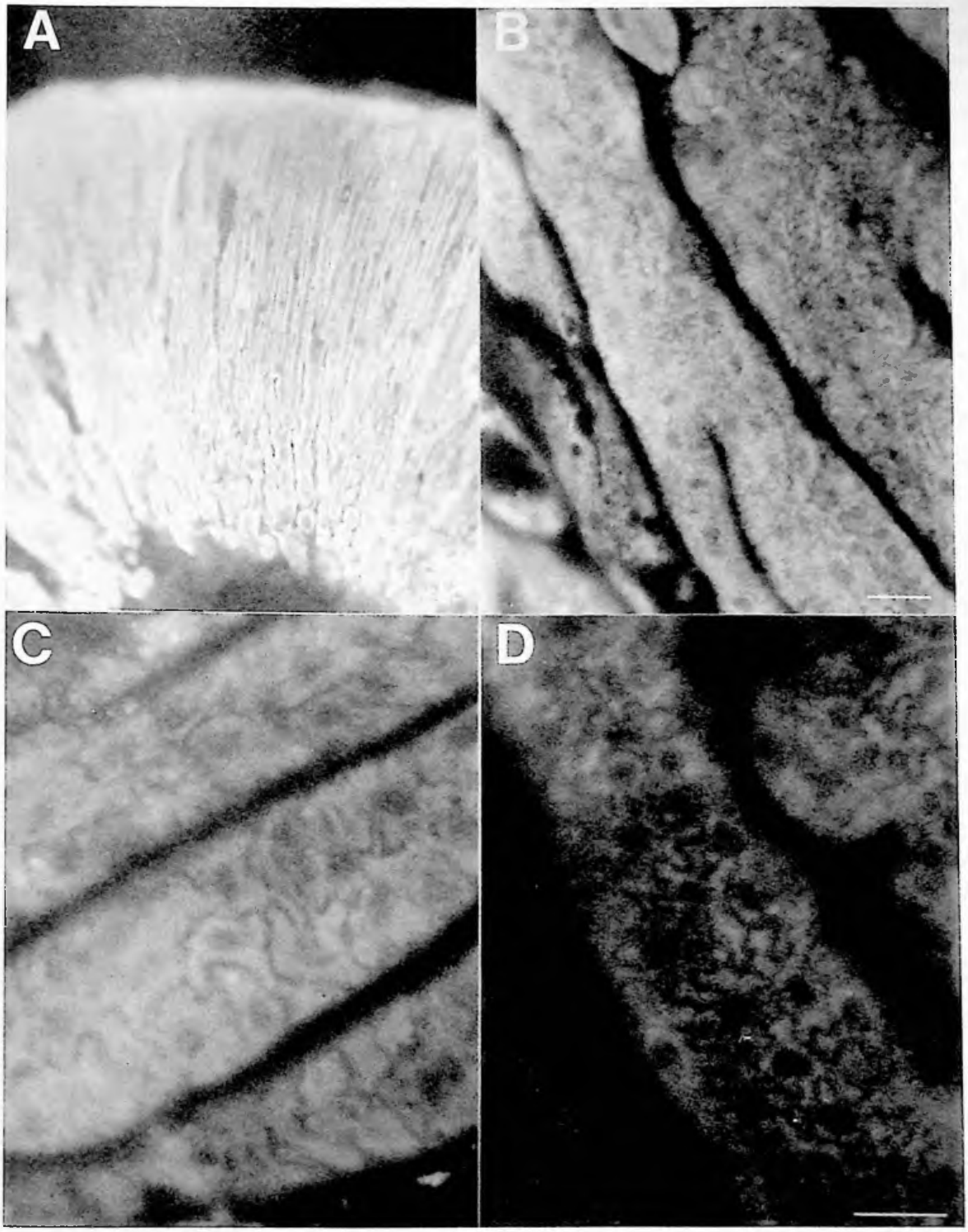

Figure 1. Rhodamine 123 staining of intact elasmobranch rectal gland. Panels A,B,C are dogfish tubules while F demonstrates skate tubules. Bar equals 100 uM in A, 50 uM in B and 25 uM in C and D.

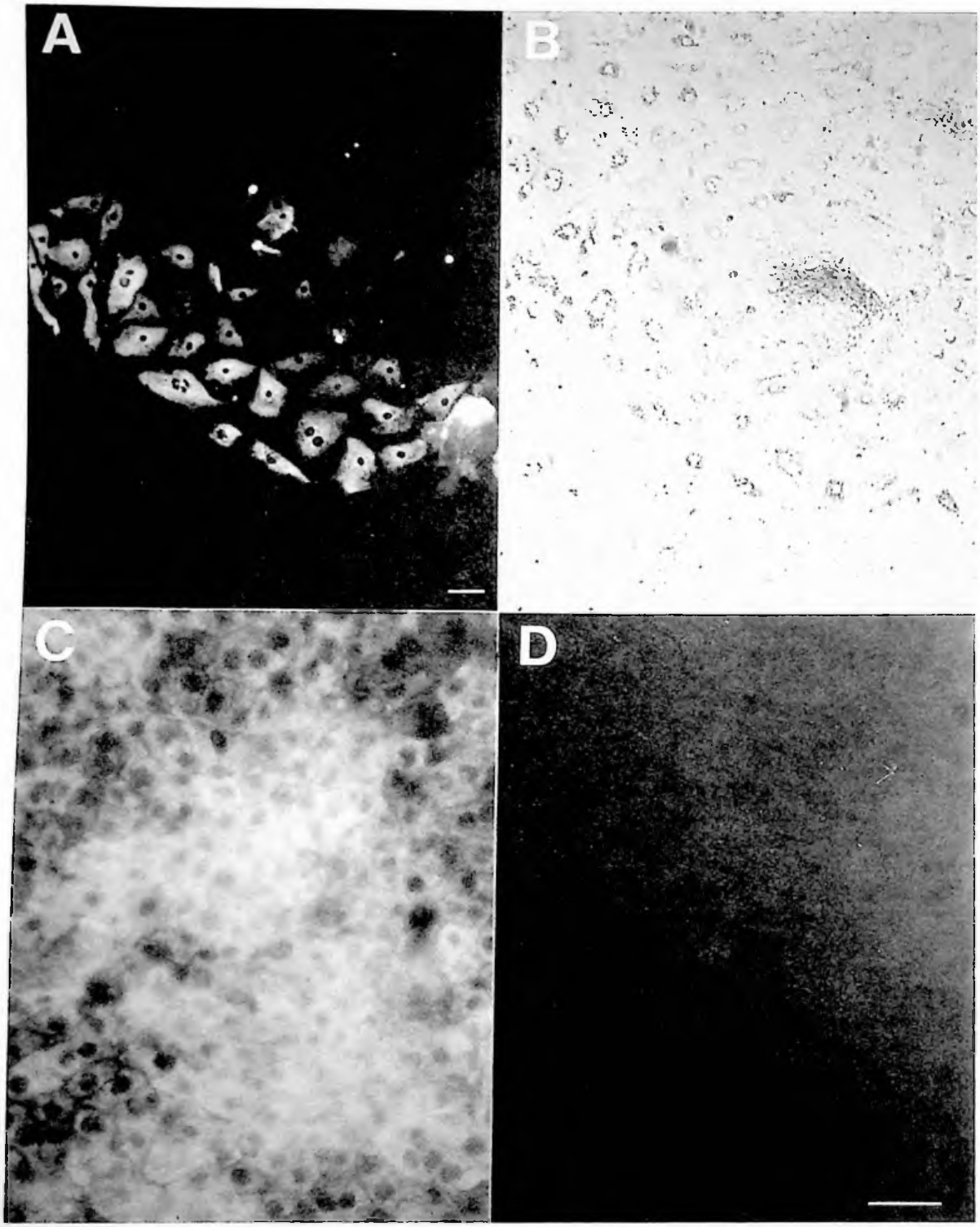

Figure 2. Rhodamine 123 staining of dogfish rectal gland cells in culture on impermeable versus permeable substrates. Panel A shows cells stained on a glass coverslip, B is the phase contrast image of A. Panel C demonstrates staining via the basolateral side in a Millicell while panel D is staining from the apical side. Bar equals 50 uM for all panels.

elasmobranch tissues to image cellular morphology. This dye may prove useful in resolution of other cell types in intact tissue. Preliminary experiments show that it is useful in demonstrating the capillary bed of the elasmobranch retina against the opaque intact sclera as well as the distinct differences in mitochondrial content along the elasmobranch kidney tubule. In addition, rhodamine 123 has been used to confirm previous data showing mitochondria in the hagfish red blood cell and their absence in the dogfish red blood cell.

Dogfish rectal gland cells in culture illustrated that caution is necessary when working with polarized cells on impermeable substates. Lipophillic rhodamine 123 does not appear to freely diffuse across all biomembranes. In experiments not shown, the diffusion of the DNA intercalating dye, ethidium bromide, also appears to be polarized in dogfish rectal gland cells. The membrane asymmetry found here is similar to that found for amino acid uptake in confluent layers of LLC-PK1 cells (Sepulveda, F.V. and Pearson, J.D. (1984) J. Cell Physiol. 118,211-217) This suggests that a number of molecular probes, substrates, labels and inhibitors thought to freely diffuse through cell membranes in an unbiased fashion may be selectively transported. Thus, substrates may cause difficulties in experimental design since only a limited population of cells will be amenable to labelling.

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