ELECTRON MICROSCOPY OF THE ALKALINE GLAND EPITHELIUM OF THE LITTLE SKATE, RAJA ERINACEA

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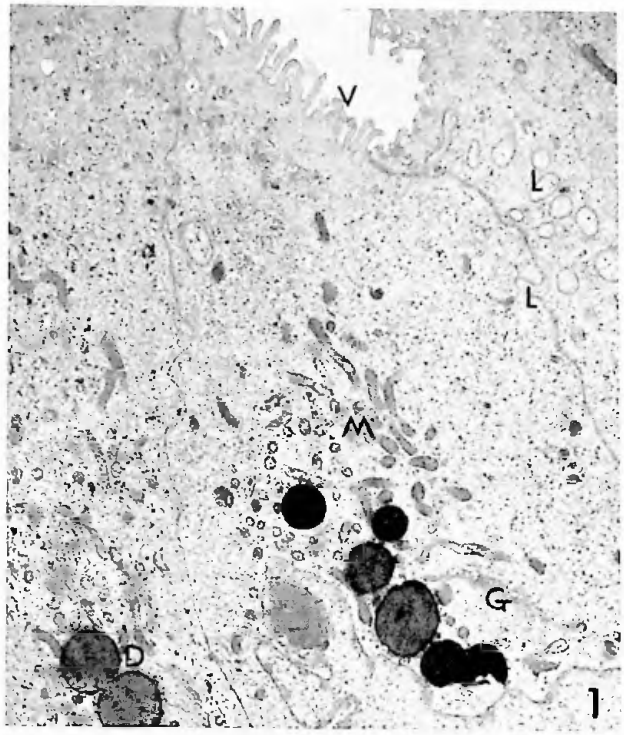
The bilobed alkaline gland of the urogenital system of the male skate is named for its pH 9.2-9.4 mucosal solution. When the gland is emptied *in situ* it requires several days to restore the 1 ml fluid content/lobe (Maren et al., Comp. Biochem. Physiol. 10:1, 1963). *In vitro* the rate of luminal alkalinization is 3.0 $\pm$ 0.2 $\mu Eq/hcm^2$ (Smith, Bulletin, MDIBL 21:80, 1981; confirmed by Kidder (personal communication)) and is the result of increased luminal $HCO_3^-/CO_3^{2-}(OH^-)$ (Smith, ibid.). Furthermore we now report that the glands of anesthetized *Raja erinacea* (0.2 ml Nembutol/kg) are capable of alkalinizing pH 6.0, HCO_3-free elasmobranch Ringers to pH 8.0 within 2 hrs while the gills are superfused with sea water (Ringers composition, mM: Na, 290; K, 5; Ca, 3.8; Mg, 3.3; Cl, 309; urea, 350; glucose, 5; buffered with 0.1 mM HPO_4-H_2PO_4).

The alkaline gland is lined by a simple columnar epithelium with apical brush border, apical basophilia, abundant mitochondria and a well-developed Golgi apparatus (Maren et al, ibid.). I began electron microscopic studies of glands fixed *in vivo* and *in vitro* to determine whether the preparation maintained the fine structural characteristics during *in vitro* incubation. The use of various fixatives has provided a subcellular differentiation of the membranes of the supranuclear region in the alkalinizing condition. The thin sections in Figs 1 and 3 were stained with uranyl acetate and lead citrate, those in 2, 4, and 5 were stained with lead citrate.

Fig 1 shows the apical microvilli (V) which compose the brush-border and the well-developed Golgi apparatus (G) in a low power electron micrograph (X 7,000) of the apical, supranuclear region of a freshly excised alkaline gland mounted briefly in an Ussing chamber and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 and post-fixed in 1% OsO_4 in 0.1 M cacodylate. Mitochondria (M) are absent from the most apical cytoplasm and are associated with the central region of the cell near the Golgi and lipid droplets (D) as well as in the basal cytoplasm (not shown). The lateral borders of adjacent cells interdigitate (L).

Fig 2. *In vitro* incubation of the alkaline gland (as in Smith, ibid.) with a constant rate of alkalinization for 150 min does not dramatically alter the fine structural components of the apical region. The gland was fixed by addition of glutaraldehyde to Ringers baths (final conc, 1% glutaraldehyde). Post-fixation with reduced osmium (1% OsO_4, 1.5% $K_4Fe(CN)_6$ in water) (Pisam, Anat. Rec. 200:401, 1981) defines two membranous systems in the apical region, seen at higher magnification in Fig 4: the apical vesicles and tubules (A) and the endoplasmic reticulum (E). Mitochondria (M), Golgi apparatus (G) and lipid droplets (D) are also seen. X 7,000,

Fig 3. The apical region of the epithelium of an *in vivo* alkalinizing gland. One lobe of the alkaline gland of an anesthetized skate was drained through the urodeum and refilled with pH 6 HCO_3^- free elasmobranch Ringers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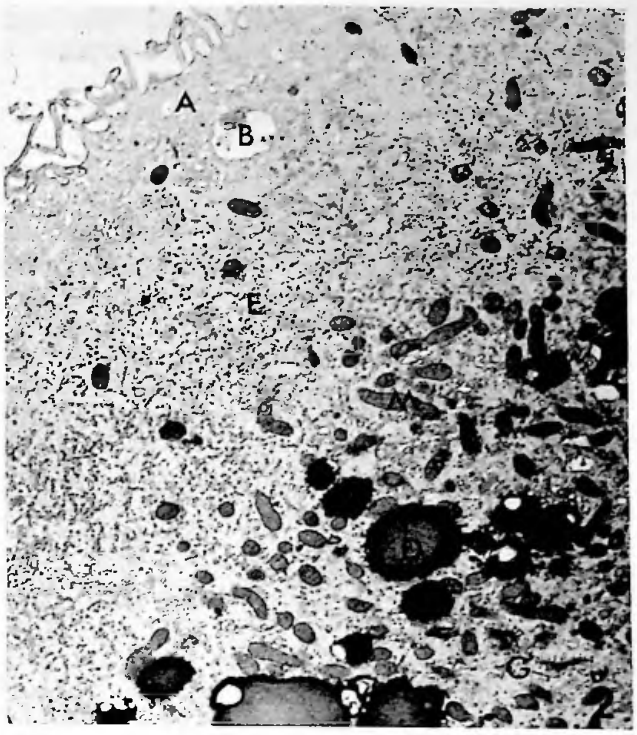
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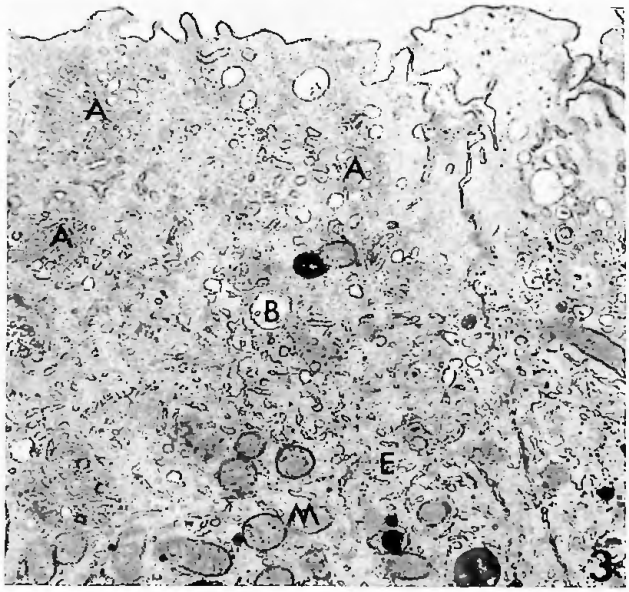
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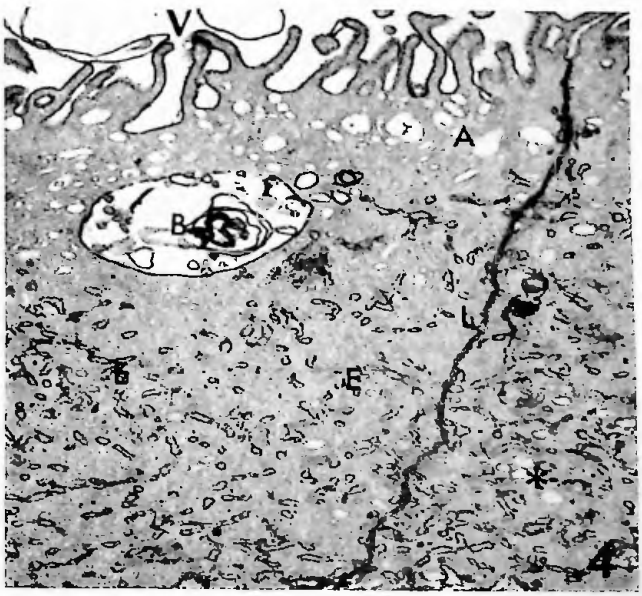
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for 15 min prior to fixation. The apical microvilli are reduced in length and number (cf. Figs.1 and 2) and there are clusters of membrane bounded vesicles and tubules (A) and one multivesicular body (MVB, (B)) in the zone apical to the ER (E) and mitochondria (M). The paired control lobes were similarly drained but refilled with alkaline fluid and fixed simultaneously with the experimental lobe; their apical cytoplasm was essentially like that in Fig 1. The glands were fixed *in situ* with 2% glutaraldehyde in 0.1M cacodylate, pH 7.2 containing 1.5% NaCl (Bundgaard and Cserr Brain Res. 226:61,1981) and post-fixed in 1% OsO_4 in 0.1M phosphate, pH 7.2.
X 13,600.

Fig 4. Electron micrograph of a 150 min *in vitro* alkalinizing gland post-fixed with reduced OsO_4 (as in Fig 2). The electron density of microvillar (V), lateral (L) and ER (E) membranes distinguishes them from the apical vesicles and tubules (A) and a few scattered vesicles within the ER (*). MVBs (endosomes) are always seen in this region of the alkalinizing gland (Cf Figs 2-5). X 19,400.

Fig 5. Ruthenium red (RR) (1% RR, 1.5% OsO , 0.067 M cacodylate) after glutaraldehyde fixation identifies vesicles (arrows) which are connected to the apical surface at the time of fixation (Chambers, J Cell Biol 57:874, 1973) (conditions as in Fig 4); the other membranes of the apical vesicles and ER are not surface connected. X 15,000.

The functional identification of these membrane systems and their dynamic relationships are worthy of further study.

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