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## Observations on the Isolated Tubule of the Dogfish Rectal Gland

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The simplicity and stability of the isolated rectal gland preparation and the ability to manipulate its extracellular fluid compartment by in vitro perfusion (Am. J. Physiol. 226: 1188, 1974) allows for the elucidation of many of the mechanisms governing secretion by this organ. Potential difference measurements across the whole gland further enhance its usefulness by providing information about the driving forces governing the movement of ions. More detailed information, however, concerning electrical and ionic conductances, unidirectional fluxes and permeability properties require an in vitro preparation in which measurements can be made across a single epithelial cell layer. The present studies were designed to investigate the feasibility of isolating single intact tubules from the dogfish rectal gland and to examine the viability of such an in vitro preparation.

Rectal glands were removed from 2-5 kg spiny dogfish, *Squalus acanthias,* and transferred to a vessel containing chilled dogfish-Ringer's solution, the composition of which was: NaCl 250, KCl 5, $MGCl_23$, $NaHCO_35$, $KH_2PO_4$ 0.5, $CaCl_25$ and urea 350 mM/L. The mesentery was removed from the outer surface and the gland transferred to a petri dish containing Ringer's solution maintained at 14-16°C and bubbled with air. Single tubules were separated by microdissection, transferred to a ceramic ring slide in a small droplet of dissection medium, and covered with a cover slip. Care was taken during the dissection to handle only one end of the tubule with the dissecting forceps in order to ensure the patency of the other end. 4-6 tubules were obtained from each gland and were examined under a microscope at a magnification of 20x to 625x.

The length of the separated tubules varied from 1-1.5 mm. The basement membrane was seen to be intact in most preparations and a patent lumen was always visible (Fig. 1). The luminal contour was irregular and was more readily defined using phase-contrast illumination. From measurements made from photographs at magnifications varying from 375x to 625x the luminal volume was calculated to constitute 6-16% of the total volume of the tubule. Individual cell borders were not distinct and the basal aspect of the cells appeared darker and more striated than the luminal aspects. There was no visible movement of fluid within the lumen and no intratubular movement was visible when a 10 l drop of Ringer's solution was allowed to flow under the edge of the coverslip.

Silva and co-workers (*Bull. MDIBL, #40 this issue*) have made the original observation that the secretory activity of the rectal gland is cyclic AMP-meditated and is greatly enhanced by the addition of theophylline to the glandular perfusate. The effect of theophylline accordingly was examined on isolated rectal gland tubules from 5 dogfish. The tubules were prepared and mounted as described in a 40 l droplet of dogfish-Ringer's. 10 l of Ringer's containing 5 mM/theophylline was allowed to flow under the edge of the coverslip (final concentration of 1 mM/theophylline) and the tubules observed at a magnification of 400 x. Within 20 seconds after addition of theophylline, streaming of fluid within the lumen was seen. Luminal diameter did not increase, but the movement of fluid was seen to cause obvious movement of the irregular luminal contour. In tubules where the dissection had caused a localized disruption of cells and overlying basement membrane, these areas were visibly widened by movement of fluid from the lumen to the exterior of the tubule. The theophylline effect was evident at 15-30 minutes after removal of the gland and attests to

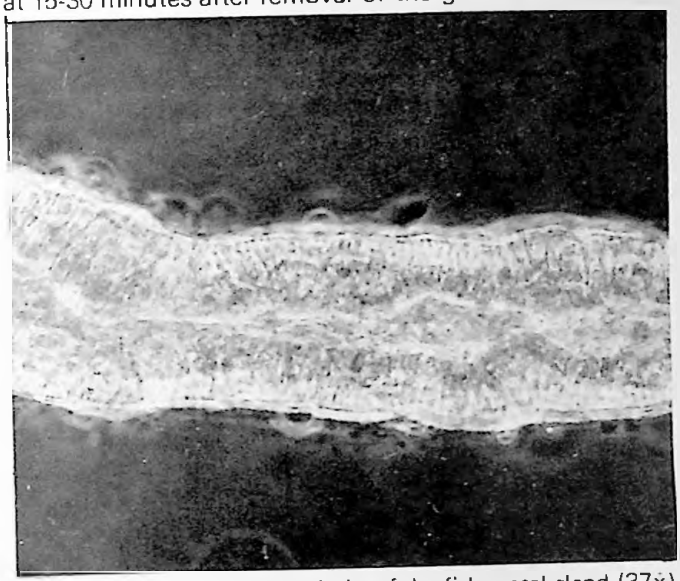

Figure 1 (above) Isolated tubule of dogfish rectal gland (37x). Phase contrast illumination reveals irregular contour of luminal border with patent lumen.



Figure 2 (below) Cross sectional view of tubule preparation used for the study of intracellular electrolytes.

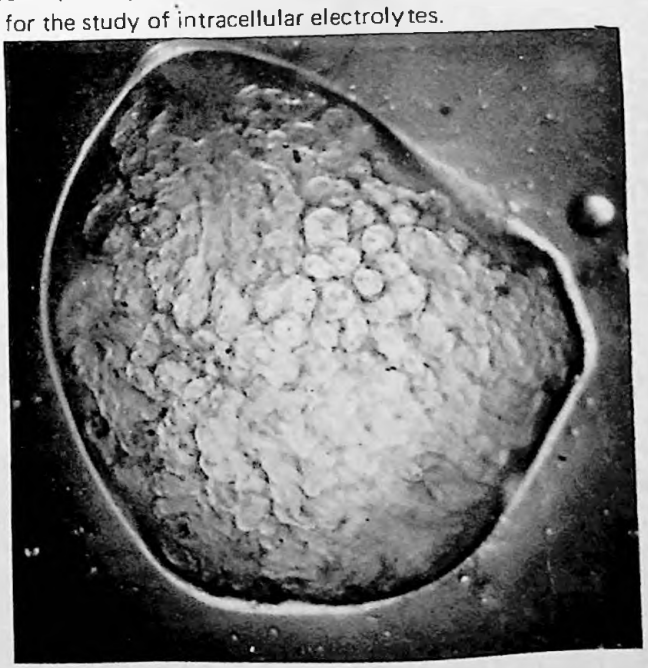

Table 1

| | Inulin Space (%) | Intracellular Electrolytes (mEq/l) (Na) | (K) | (Cl) |
|---|---|---|---|---|
| Mean | 29.00 | 42.1 | 161.6 | 116 |
| SD | 1.69 | 8.4 | 19.1 | 23.7 |

the viability of the preparation.

The presence of a patent lumen (even in glands unstimulated by theophylline or dibutryl cyclic AMP) containing fluid of very high Na and Cl content was thought to complicate the interpretation of intracellular electrolyte measurements made on whole tissue. The following preparation permitted the free entry of labeled inulin into the lumen, and correction for this extracellular compartment.

Freshly harvested rectal glands from 5 dogfish were sectioned by means of a Stadie-Riggs microtome so that the individual glandular tubules were cut in cross section (Fig. 2). Sections approximately 200 in thickness and 4-5 mm in diameter were then observed under a dissecting microscope to ensure that the direction of sectioning was correct; lumina of the sectioned tubules appeared to be patent, giving a "doughnut-like" appearance. The sections were then transferred to conical flasks where they were incubated at 15°C, with air bubbling, in 4 ml of dogfish Ringer's to which $^{14}C$ inulin (50 ci/ml) was added. Three sections from each gland were incubated for 15 minutes and another section for 30 minutes to ensure complete labeling of the extracellular space. For the determination of total extracellular space, sections were removed from the flasks, blotted dry on filter paper, weighed, placed in counting vials and extracted in 0.4 ml NCS tissue solubilizer (amersham/Searle Corp., Ill.) for 24 hours. The tissue was then reweighed. For Na and K determinations the tissue was digested in 100 l of concentrated nitric acid for 24 hours, and Na and K concentrations measured using a flame photometer. For chloride determination, the dried tissue was boiled in 100 l of deionized water in sealed tubes for 2 hours and chloride concentration measured using a Cotlove chloridometer.

The results of the above measurements are depicted in Table 1. The measured inulin space of 29±1.69% of wet tissue weight is slightly, but significantly greater than the value of 26.7±1.2 obtained in vivo by Silva et al. (*Bull. MDIBL, 14: 116, 1974*). There was no difference between the inulin space determined at 15 and 30 minutes incubation and only the former values are listed. intracellular electrolyte concentrations agree reasonably closely with the previously reported in vivo measurements of Silva et al; however, both intracellular Na and Cl concentration are higher in the present studies.

These studies demonstrate that in vitro studies on isolated tubules of the dogfish rectal gland are possible and that further useful information may be derived using the technique of in vitro perfusion of single tubule segments.

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## The Electrical And Hydrophilic Properties Of Fish Cornea

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The transparency of the cornea is linked to th optical properties of its connective tissue layer, called th stroma. The optical properties depend on the degree c hydration of the stroma, and the hydration is contro e by ionic pumps located in the epithelium and endothelu of the cornea. There is ample evidence for the propert e of the cornea of mammalians and amphibians, howeve studies extending to the lower vertebrates and inve tebrates have been less frequently reported.

We have examined the electrical properties, thic ness and hydration characteristics of the corneas of a elasmobranch, the spiny dogfish, *Squalus acanthias* a some teleosts, the sculpins' *Myoxocephalus octodece spinosus* and *Myoxocephalus scorpius*, and one specim of the cod fish, *Gadus callarias*. The background to the studies is that sharks' corneas are known not to swell c become opaque. On the other hand, the cornea c teleosts is known to swell and in this sense they a similar to amphibians and mammalians. (Smelser, Inves Ophthal, 1:1, 1962). However a detailed study of th thickness and electrical properties of these fish cornea does not exist. In the case of the shark cornea, previo attempts to determine the existence of any electric potential difference across it, when placed as membrane in a lucite chamber showed that the resistan was extremely low. In fact, we have found that this is du to a lesion of the corneal epithelium produced durin collection of the specimens, because of rubbing of th corneal epithelium against the sand paper like skins of th companion dogfish in the catch. Appropriate protectio was provided, by harnessing the recently caught dogfis with a protection made of metal wires and rubber tha prevented lesions to the cornea. The protective harness shown in Figure 1. The lesion of the epithelium is ea

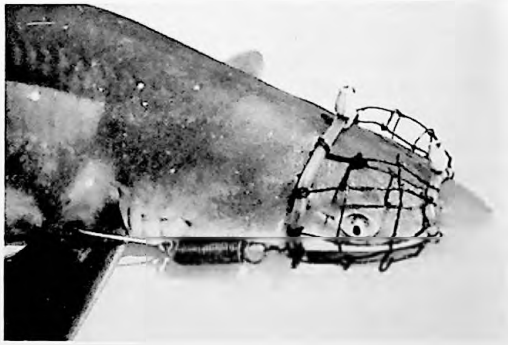

Figure 1

observed if a few drops of fluorescein are instilled in th eyes of the fish. This classical test shows the geographi borders of the lesion of the epithelium where fluoresce penetrates. Figure 2 shows the lesions produced i unprotected animals. These lesions do not occur in th protected eyes. The values for potential differences an resistances observed in protected corneas, unprotecte and others handled specially are shown in Table I. Th corneas were disected out and mounted in special lucit chambers for corneal research of an aperture of 1 cm.2 i is clear that the results that approximate more to th