HISTOLOGY OF THE RECTAL GLAND OF *Squalus acanthias*

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Although the histology and some aspects of the fine structure of the rectal gland have been described previously; Hoskins, J. Morph. 28:329 (1917); Bulger, Anat. Rec. 147:95 (1963); Crofts, Proc. Roy. Soc. (London) Pt. 1:101 (1925); Komnick et al. Ztschr. Zellforsch. 74:123 (1966); and others; recent physiological experiments on the isolated gland have prompted a review of the histology and some revisions based on electron microscopy. Some effects of stimulation and inhibition are noted by Doyle, Bulletin MDIBL 15:28 (1975). The following summary is based on a reexamination of light microscopic and electron micrographic preparations and for sake of brevity does not identify all prior observations by others.

These observations are based on glands taken from normal animals and from isolated, stimulated, and inhibited rectal glands of *Squalus* collected at MDIBL, fixed at measured functional states, and fixed by arterial perfusion with modified Karnovsky fixative (2% glutaraldehyde, 4% paraformaldehyde in 0.1 M cacodylate buffer pH 7.2), perfused at 50 cm pressure against an open vein.

General Anatomy: The rectal gland lies dorsal to the spiral valve of the intestine enveloped by its mesentery; the excretory duct of the gland entering the postvalvular intestinal wall in a cephalad direction. The rectal gland artery arises from the dorsal aorta and runs within the mesentery to be distributed within the capsule of the gland by major dorsal longitudinal branches, some smaller circumferential arteries and a ventral longitudinal artery. Each of these vessels gives rise by profuse branching to thin walled arteries within the capsule. As described below these capsular arteries give rise to sinusoidal vessels running to the central canal where they enter a large venous sinus occupying the ventral half of the central canal. This sinus leads to the dorsal intestinal vein on the spiral valve.

The parenchyma of the gland consists of radially arranged secretory tubules which are surrounded by sinusoidal vessels. The flow of rectal gland secretion is thus concurrent to the blood flow. The secretory tubules end at the central canal by an abrupt transition of epithelium to form the excretory duct consisting of a stratified (3 layered) epithelium surrounded by a connective tissue layer containing nerves and smooth muscle and an outer boundary of blood sinus endothelium.

A fairly large mixed nerve supplies the gland and is observed in the capsule and as unmyelinated axons in the wall of the excretory duct ending on smooth muscle. Nerve terminals have dense cored vesicles.

The following observations are based primarily on sections at the midlevel of a gland 6 mm in diameter.

Capsule: The gland is surrounded by a capsule about 0.1 mm thick consisting of the mesentery, a collagenous connective tissue containing myelinated and unmyelinated nerves, circular and longitudinal smooth muscle and thin walled arterial vessels (capsular arteries) most of which are about 50 μm in diameter. The mesentery is composed of cuboidal cells whose free surface is adorned with ridges (microplicae) and, at the apical surface, a single layer of mucus vesicles thus providing a continuous mucous epithelium without goblet cells. From other studies on the intra-peritoneal injection of a variety of drugs, this peritoneal lining must be considered rather impermeable. The adjacent borders of peritoneal cells have classical junctional complexes (presumably tight).

Parenchyma: The parenchyma of the gland consists of secretory tubules and blood sinusoids. The outer third of the parenchyma consists of closely packed tubules and the inner third of confluent sinusoids surrounding tubules well separated from each other. The secretory tubules consist of a single cell type with no mucous (goblet) cells. The tubules branch in their course from the central canal to the periphery and although Hoskins described six orders of tubules we cannot confirm this. In our glands of 6 mm diameter the central canal averaged 1.6 mm in diameter and the parenchyma 2.2 mm on each side of it. The tubules are a little longer than 2.2 mm since at the canal some of them run slightly caudad. At the periphery the tubules are fairly uniform in diameter with lumens 3-5 μm in diameter, these tubules run radially unbranched for

about 0.25 mm. By fusion of some of the tubules at various levels of the gland (notably at 0.25, 0.6, 1.1 and 1.4 mm from the periphery) the number of tubules at the central canal is reduced four fold. Although we can distinguish 4 orders of tubules (3 fusions) for some tubules, others may have fused only once or twice so that there is an admixture of tubules with large or small lumens even at the central canal where the average diameter of the lumen is about 50 μm.

We have found about 512 tubules at the periphery of the gland and about 64 surrounding the central canal so that there are on the average 8 peripheral tubules for each central (primary) one. The length (and diameter) of the gland varies with the individual fish but assuming a gland 3.5 cm long with peripheral tubules spaced 35 μm apart we would find 512,000 peripheral tubules and 64,000 primary tubules entering the excretory ducts. Not all secretory tubules branch at the same level nor for the same number of times. From measurements of lumen diameters we conclude that the total lumenal volume has increased at least 4.5 times en route to the central canal and that there is little difference in this volume in fixed preparations taken from theophylline stimulated and ouabain inhibited glands.

Blood sinusoids - There is very little connective tissue between the secretory tubules and the sinusoids of the parenchyma and only very few collagenous strands extend from the capsule. Beneath the capsule the thin walled capsular arteries give rise usually directly to sinusoids of about 10 μm diameter but occasionally to subcapsular cisterns about 150 μm in diameter from which many small sinusoids arise. Sections across the tubules at the periphery show the tubules closely packed with blood sinusoids between them in such a way that the bases of about half the tubule cells abut on other tubules and about half on sinusoids. This close packing extends about 0.4 mm from the periphery at which level one finds some large (fused) tubules intermixed. At 1.5 mm from the periphery the tubules are about 95% surrounded by sinusoidal walls. From 1.8 mm to the central canal large secretory tubules predominate and adjacent tubules are only intermittently in contact with other tubules; the surrounding sinusoidal space being about 35 μm across. The sinusoidal endothelium is extremely thin, usually 0.2 μm thick, but varying from 0.03 μm to 0.5 μm except where the nuclei are present (2-3 μm). Despite this thinness we have found no fenestrae.

KINETIC PROPERTIES OF RED CELL CARBONIC ANHYDRASE IN *S. acanthias* AND *L. americanus*, IN RELATION TO THE VERTEBRATE PHYLOGENY OF THE ENZYME

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Primate red cells contain two isoenzymes of carbonic anhydrase, with very similar active sites and tertiary structure, but only 60% homology in amino-acid sequence. The enzyme designated C is some 20-fold more active than B, although the latter is about 5 times more abundant, being (at 4 g/liter RBC) the second most concentrated protein in red cells. Analysis of enzyme patterns in other mammals, as well as in birds and amphibia, suggest that a high activity enzyme, akin to C, is always present in red cells. Most mammals have both types, although certain ones (dolphin, sheep, ox, cat) have only C. All submammals thus far examined have but a single enzyme, and kinetic analysis in the frog and chicken, for example, show it to be akin to C (Bundy, Comp. Biochem. Physiol. 57B:1, 1977). In human populations, C is far more stable in concentration and iso-electric pattern, while B is variable and occasionally absent (Kendall and Tashian, Science 197:471, 1977). No function has yet been found for B; the very powerful catalytic activity of C provides a generous reserve for respiratory events, including exercise to exhaustion (Maren and Swenson, Federation Proc. 1978).

These and other considerations have suggested that C may be the archtypal enzyme, with gene duplication occurring some 10^8 years ago (Tashian and Carter, in Advances in Human Genetics, Vol. 7, Plenum Press, 1976). Only one study has been made on the kinetics of fish carbonic anhydrase, that of Maynard and Coleman