32 FUNCTIONAL MORPHOLOGY OF THE FLOUNDER GLOMERULAR CAPILLARY WALL

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The peptide antifreezes of the winter flounder are synthesized seasonally in preparation for winter environmental conditions (Petzel et al., J. Exp. Zool., 211:63, 1980). The antifreeze peptides are low molecular weight (3 to 5 thousand daltons) anionic (pI 4.1 to 4.5) peptides which reach peak serum concentrations of 3 to 5 percent. We have proposed that these anionic peptides are conserved in the circulation through repulsion from the polyanionic glycosaminoglycans of the glomerular capillary wall (GCW). This proposed mechanism of antifreeze peptide conservation is similar to that of albumin by the mammalian kidney (Purtell et al., Kidney Int. 16:366, 1979). Upon modifying the free carbonyl groups of the peptides with lysine there was an increase in the isoelectric points of the peptides and after i.v. injection, an 8.3 to 8.5 fold increase in the clearance of the cationized derivative over the native (anionic) antifreeze peptides (Petzel and DeVries, Bull. MDIBL, 20:17-18, 1980, and Table 1). This suggests that the GCW possesses an anionic charge which repells the anionic antifreeze peptides but attracts the cationic from leading to increased glomerular filtration of the lysine modified peptides.

Table 1. Renal clearances of polyethylene glycol (PEG), anionic antifreeze peptides (AAP) and neutral antifreeze peptides (NAP) in the winter flounder *Pseudopleuronectes americanus*. (see Petzel and DeVries, Bull, MDIBL 20:17, 1980 for methods)

Substance Injected	n	Weight (kg)	U/P	Clearance ml/hr/kg	Fractional Clearance
PEG	6(51)*	.30(.02)**	3.98(.93)	2.21(.23)	1.00
AAP	2(25)	.47(.08)	0.19(.01)	0.04(.01)	0.02(.01)
NAP	5(35)	.29(.03)	0.51(.07)	0.37(.07)	0.17(.03)

*n = number of fish (number of 10 hour clearance periods)

**mean of fish ± S.E.

To confirm this thesis of a charge to charge repulsion mechanism we characterized the structure and localized the anionic charges of the winter flounder GCW through electron microscopy using cationized ferritin as a tracer.

Winter flounder weighing 250 gm were injected intravenously with native (pI 4.1-4.6) and cationic (pI > 8) ferritin (SIGMA) at a dosage of 10 mg per 100 gm body weight. The fish were allowed to swim freely for 60 min., after which the kidney was excised and immediately immersed in fixative (4% glutaraldehyde, 2% paraformaldehyde, 2.5 mM $CaCl_2$ in 0.2 M cacodylate, pH 7.4). Kidney samples for light microscopy were dehydrated in EtOH, embedded in paraplast, sectioned and stained in hematoylin and eosin. The mean diameters of the glomerulus and Bowman's capsule were taken from glomeruli showing the vascular pole. Tissue for electron microscopy was post-fixed in 1% OsO_4 in 0.2 M cacodylate buffer and dehydrated in EtOH. Secondary electron scanning micrographs were prepared from tissues critical point dried in EtOH, sputter coated with gold and imaged on a JEOL JSM U-3 at 10 KeV. Tissue for transmission electron microscopy (TEM) was embedded in EPON 812, sectioned, stained with aqueous uranyl acetate and lead citrate and imaged on a

JEO 100 C at 100 KeV. The morphometrical data presented was taken from negatives after magnification calibration of the 100-C.

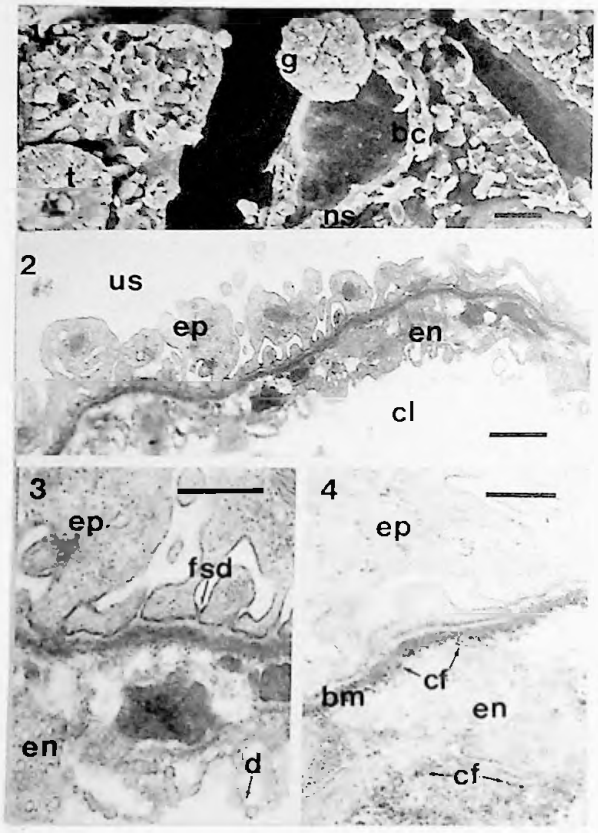

Figure 1.--Scanning electron micrograph of winter flounder kidney. bc = Bowman's capsule, g = glomerulus, ns = neck segment of nephron, t = renal tubule, scale bar = 25 μm, mag. = 230 x.

Figure 2.--Survey TEM micrograph of GCW from fish injected with native ferritin, stained with uranium and lead. cl = capillary lumen, en = endothelium, ep = visceral epithelium, us = urinary space, scale bar = 1 μm, mag. = 7,600 x.

Figure 3.--High magnification of Figure 2 showing endothelial diaphragm (d) and filtration slit diaphragm (fsd) between interdigitating pedicels epithelial podocytes, scale bar = 0.5 μm, mag. = 23.150 x.

Figure 4.--High magnification of GCW from fish injected with cationized ferritin, stained with uranium only. Cationized ferritin (cf) is bound to the basement membrane (bm), scale bar = 0.5 μm, mag. 18,600 x.

The renal corpuscles studied were sampled ventral to the major artery running along the posterior dorsal surface of the kidney. From light and scanning microscopy, the glomerulus is bordered by interstitial and tubular tissue. The glomerulus is surrounded by the parietal epithelium of Bowman's capsule which in turn tapers to the neck segment of the nephron (Figure 1). The average diameter (± S.E.) of the glomerulus was 58.5 ± 1.9 μm and that of Bowman's capsule 69.4 ± 2.5 μm. Imaging with the TEM reveals that the GCW possesses endothelium with fenestrae occluded by a diaphragm, mesangium, interditating epithelial podocytic pedicels spanned by a filtration slit diaphragm and the glomerular basement membrane (Figures 2 to 4). The thickness of the basement membrane varied from 70 to 100 nm, which is similar in magnitude to that of mammalian glomerular basement membranes. Areas of the basement membrane which were bordered by the fenestrated endothelium on the blood side and the pedicels on the urinary side with endothelium or mesangium between the fenestrae and the basement

membrane were common. This increase in the thickness of the filtration barrier has been reported in other salt water teleosts (Olsen, Acta Pathol. Microbiol. Scand. 212:815, 1970; Bonga, Z. Zellforsch. 137:563, 1973).

Glomeruli from fish injected with either native or cationized ferritin resulted in retention of the tracer by the GCW as evidenced by the lack of ferritin accumulation in the urinary space, tubular lumen and tubular epithelial cells (Figures 2 to 4). The cationized ferritin accumulated in clusters along endothelial cell membranes and the blood side of the glomerular basement membrane (Figure 4). No such binding of the native ferritin was observed. This binding of the positively charged ferritin to the GBM confirms the anionic nature of the GCW.

Thus we conclude that the anionic antifreeze peptides are retained in the circulation through their repulsion from the anionic GCW of the winter flounder. This research was supported by a University of Illinois Graduate College Dissertation Research Grant (to DHP) and NSF Grant PCM 77-25166 (to ALD).

27 TOPOGRAPHIC DISTRIBUTION OF STEROIDOGENIC ENZYMES IN SQUALUS TESTIS: STRUCTURAL AND FUNCTIONAL CORRELATIONS.

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The role of steroids within the testis remains a problem of great interest in the field of male reproduction. It is generally agreed that androgens derived primarily from interstitial Leydig cells are required at some stage of spermatogenesis, and androgen receptors have recently been located in both Sertoli cells and germ cells. A less well-known fact is that estrogen (formed by aromatization of androgen) is a normal product of the testis and, in certain species, comprises a large proportion of total testicular steroid secretion (e.g., stallion, boar, Necturus). Because estrogen receptors are located in Leydig cells and several enzymatic steps leading to androgen biosynthesis are inhibited by estrogen, it seems likely that it is part of a short feedback loop governing further androgen output. Androgens and estrogens are therefore interrelated biosynthetically and functionally. There is some controversy over whether Leydig cells or Sertoli cells are the primary source of intratesticular estrogen.

Most of what we know about testicular functions has been obtained from studies in laboratory mammals. In these species, all stages of germ cell development are present simultaneously from the onset of puberty. Moreover, their gross distribution throughout the testis is uniform, although on a microscopic level it can be seen that only certain stages occur together (specific cell associations) in any one segment of the semiferous tubule. Using these species it is technically difficult, without disrupting the normal relationship between the tubules and interstitium, to study how the steroidal microenvironment might change during the cycle of the seminiferous epithelium. Certain nonmammalian vertebrates would seem to be natural models for investigating this problem. In the shark, for example, germ cells in different stages of development are topographically segregated within the testis. In earlier studies we found that Squalus testis synthesized substantial amounts of estrone and estradiol-17β from ^{3}H-androgen (Callard, G.V., et al., Endocrinology 103:2283, 1978) and that aromatase activity was greatest in microsomes prepared from regions in which germ cells were undergoing meiotic divisions (Callard, G.V., and Petro, Z. The Bulletin, MDIBL 19:38, 1979). The purpose of the present investigation was to expand our study of steroidogenesis to include other key enzymes leading to androgen and estrogen bio synthesis and, using light and electron microscopy, to characterize the steroid-secreting cell types.

The testes of male sharks captured in July were sectioned transversely and each slice further separated into 3 regions based on the following criteria: opacity; color; position in relation to the epigonal tissue. Tissues were homogenized in sucrose/phosphate buffer, and microsomes prepared by centrifugation (100,000 x g x 60 min) were resuspended in phosphate buffer. Whole homogenates of free spermatozoa collected from the vas deferens