CHARACTERIZATION AND STAGE-DEPENDENT DISTRIBUTION OF AN ESTROGEN SULFOTRANSFERASE IN THE TESTIS OF THE DOGFISH SQUALUS ACANTHIAS.

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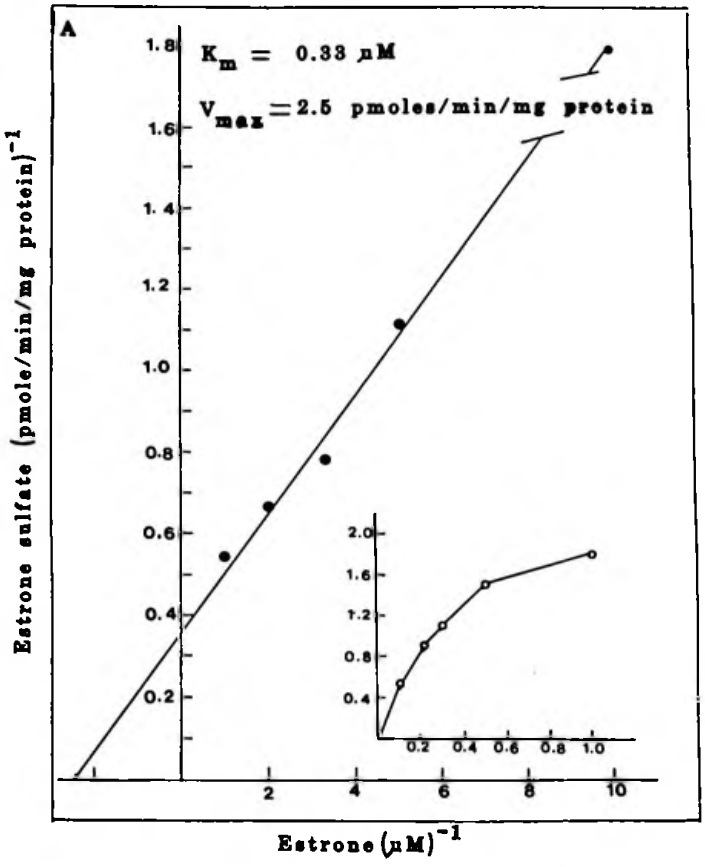
In the spiny dogfish *Squalus acanthias* germ cells in successive stages of maturation and their associated endocrine elements are topographically separated (Callard *et al.*, Endocrinology 117:1328-1335, 1985). This allows us to obtain tissues from discrete stages for biochemical analysis of steroidogenic enzymes and steroid receptors. Using this approach, we have demonstrated that (1) enzymes of androgen biosynthesis are maximal during the final stages of spermatogenesis (Zone III, fully mature spermatozoa); (2) enzymes of estrogen production peak during meiosis (Zone II, primary and secondary spermatocytes); and (3) androgen and estrogen receptors are localized in regions with stem cells and spermatogonia (Zone I). In *Squalus*, blood first enters the testis via Zone III, passing successively through Zones II and I; therefore, we postulated a pathway of communication from more mature to less mature regions with steroids serving as parahormonal regulators. In preliminary experiments, we observed high yields of sulfoconjugated estrogens after perfusion of the testis with [^{3}H]estradiol or [^{3}H]testosterone. Since sulfated steroids are incapable of receptor binding, the sulfotransferase system may be part of a mechanism for regulating the availability of biologically active hormone in discrete target areas. We report here the reaction properties and stage-dependent distribution of an estrogen sulfotransferase in *Squalus* testis.

Testes were dissected into three zones (see above), homogenized in sucrose-phosphate buffer (250/50 mM, pH 7.4) and cytosols obtained by centrifuging at 100,000 x g x 60 min. Sulfotransferase activity was measured in cytosol incubated in 0.1 M Tris buffer (1 ml, pH 7.4) containing 35 mM monothioglycerol, 50 μM of the active sulfate donor phosphate adenosine phosphosulfate (PAPS) and 1 μM [^{3}H]estrone as substrate at 22 C. The reaction was stopped by extraction with chloroform (4 ml) and [^{3}H]estrone conjugates measured in the aqueous solution.

Using 1 μM estrone and 0.5 mg cytosolic protein per assay tube, product yields were linear for 90 min. An experiment in which steroid substrate was varied between 0.1 and 1.0 μM showed that the enzyme was saturated at 1.0 μM. A Lineweaver-Burk plot indicated normal Michaelis-Menton kinetics and the following reaction constants: Km = 0.33 μM Vmax = 2.5 pmoles/min/mg protein (Fig. 1A). Experiments in which the cofactor concentration was varied and steroid substrate kept constant (1 μM) gave a Km for PAPS of 1.07 μM and a Vmax of 1.5 pmoles/min/mg protein) (Fig. 1B). Substrate preference was tested by incubating 1 μM [^{3}H]estrone with one- or five-fold excess radioinert competitor. Estrogens were preferred substrates, although androgens were also effective (estradiol> estrone> dehydroepiandrosterone = testosterone> 5α-dihydrotestosterone); estriol was a poor competitor (Fig. 2). When the testis was separated into three zones based on germ cell composition, sulfotransferase activity was highest in Zone III containing fully mature spermatozoa (2.4 ± 0.24 pmoles/min/mg protein) with no difference between the two less mature zones (1.22 ± 0.22 and 1.28 ± 9.21 pmoles/min/mg protein) ($p<0.01$) ($n = 3$).



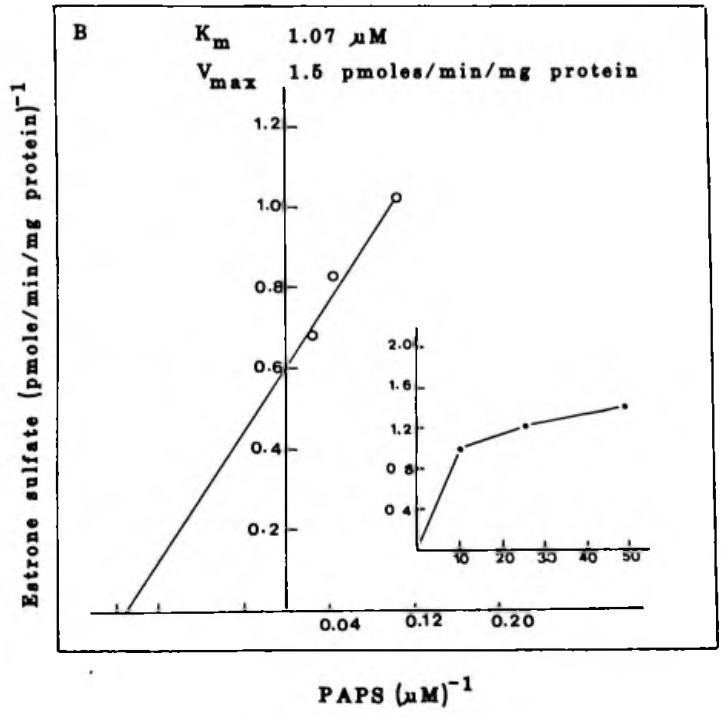


Figure 1. Lineweaver-Burk plots and saturation analyses (inserts) of estrone sulfate production when steroid substrate (estrone) concentration is varied (A) and when the active sulfate donor (PAPS) concentration is varied (B).

The reaction properties of the estrogen sulfotransferase in Squalus testis are similar to those reported for mammalian tissues; however, specific enzyme activity in Zone III is 42 times higher than that in whole rat testis, indicating that conjugation is a more important pathway of steroid metabolism in an elasmobranch. Although estradiol is the preferred substrate and we have designated this enzyme an estradiol sulfotransferase, androgens are also conjugated. Whether this reflects a mixture of estrogen and androgen sulfotransferases or a single enzyme that recognizes both steroid classes cannot be determined with the crude cytosols used in the present study. The Sertoli cell is the primary steroidogenic element in Squalus testis and is most highly differentiated in Zone III (Pudney and Callard, Anat. Rec. 209:311-330, 1984), thus corresponding to the zonal distribution of conjugating activity. Additionally, this distribution suggests that sulfotransferase is ideally positioned to negate the effects of circulating estrogen which enters the testis via Zone III. If so, occupancy of estrogen receptors in Zone I would depend entirely on estradiol synthesis in adjacent Zone II where aromatase is known to be maximal. Likewise, this sulfotransferase would inactivate circulating androgen; however, high levels of androgen biosynthetic enzymes present in Zone III may provide sufficient androgen substrate for aromatization in Zone II and for androgen receptor binding in Zone I. Results of perfusion experiments support this interpretation (Cuevas, Miller and Callard, unpublished). These preliminary studies lead us to conclude that steroid sulfotransferase regulates access of circulating hormone to the testis and reinforces the view that spermatogenesis is controlled exclusively by androgen and estrogen synthesized in situ (paracrine control).

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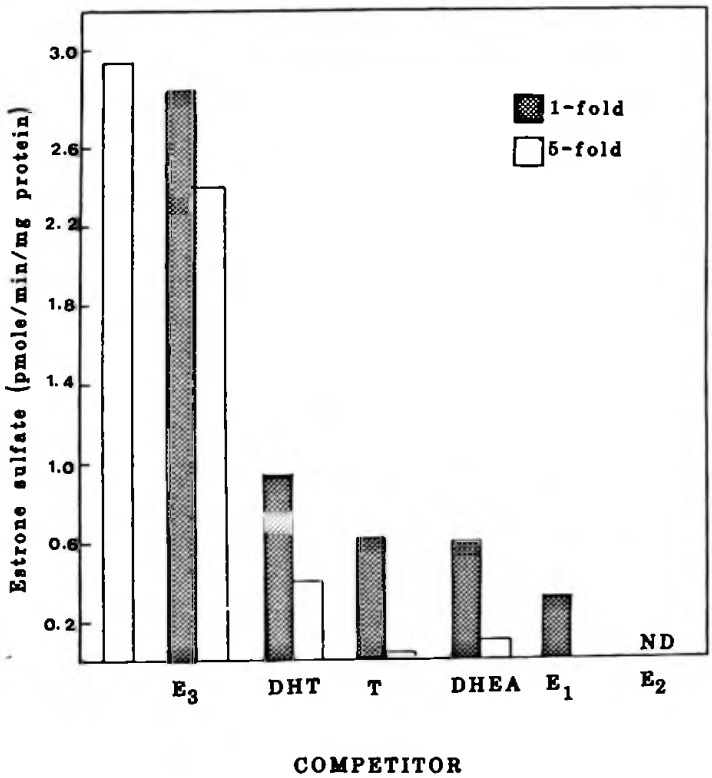

Figure 2. Substrate specificity of sulfotransferase as determined by inhibition of [^{3}H]estrone sulfate production in the absence (first clear bar on left) or presence of 1- or 5-fold excess radioinert competitor. E_1, estrone; E_2, estradiol; E_3, estriol, T, testosterone; DHT, 5α-dihydrotestosterone; DHEA, dehydroepiandrosterone.