transporter. The reduction in s-m flux, and the abolition of its furosemide sensitive component by the serosal substitution of impermeant ions for Na or Cl places this transporter on the basolateral border of the tissue.

We have previously shown that the accumulation within the epithelial cell and the secretion of 2-d-gal is sensitive to removal of either serosal K or Cl or addition of furosemide to the serosal bathing solution (R.J. Naftalin, K. Thompson and A. Kleinzeller, Bull MDIBL 21:62). This indicates some linkage between the serosal border Na-K-Cl cotransporter and 2-d-G secretion.

Further evidence of an interdependence between the s-m flux of sugar and ^{86}Rb may be seen as the serosal addition of 10 mM 2-d-gal causes a significant rise in the S-M flux of ^{86}Rb. This phenomenon lacks specificity, in that 10 mM mannitol, which is not secreted by the flounder causes a similar rise in ^{86}Rb flux.

Current research into ion transport across the rabbit ileum had demonstrated that the submucosal compartment of this tissue has an important role in the regulation of the access of the basolateral border to salts and water. The effect of mannitol and 2-d-G on transport may be explained by an increase in the access of the basolateral border to ^{86}Rb possibly resulting from an increased unfolding of the basolateral membrane. This will lead to an increased uptake of 2-d-G into the cells as it has been previously been shown (R.J. Naftalin and A. Kleinzeller, Am. J. Physiol. 240:G392-G400) that the basolateral membrane is highly permeable to this sugar. This work was supported by grants N.A.T.O. to R.J.N. and P.M.S. and from the Royal Society and Dale Fund to P.M.S.

IN VITRO STEROID PRODUCTION BY OVARIAN GRANULOSA CELLS OF SQUALUS acanthias

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The reproductive cycle of the spiny dogfish, Squalus acanthias, is 22 months in length (Hisaw and Albert, Biol. Bull. 92-93:187-199, 1947). There are four identifiable stages of pregnancy (A through D); each one is associated with changes in embryo size and ovarian follicular development. We have already reported that isolated ovarian follicular cells of another elasmobranch, Raja erinacea, have steroidogenic potential (Tsang and Callard, Bull. MIDBL 22:96, 1982). The current study provides an analysis of the steroidogenic potential of isolated granulosa cells from the ovary of Squalus acanthias, and changes in steroidogenesis associated with follicular growth during gestation.

Animals were kept in recirculating sea water tanks until use. After spinal pithing, ovaries were excised and kept in cold buffer. The follicles were separated, measured and the stage of gestation noted. Following scissor puncture, the yolk was expressed from each follicle, and the remaining follicular envelope rinsed several times with buffer. The granulosa layer was separated from the theca and dispersed with collagenase. The cells were isolated by low speed centrifugation (800 x G) and were finally resuspended in basal medium containing Eagle's salts, urea and glutamine. Aliquots containing 250,000 cells were incubated for 4 hours at 18°C. The medium was collected and the progesterone (P), testosterone (T) and estradiol (E) content was determined by radioimmunoassay.

Granulosa cells of stages A, B and C animals produced negligible amounts of P, while those from stage D animals were capable of producing large amounts (Table 1). Coupled with the fact that circulating levels are high during early pregnancy (Tsang, unpublished), this supports the idea of an extra-follicular source of P, with the most likely one being the corpus luteum.

Testosterone production is non-detectable in stage A animals, and levels remain low until the end of gestation. Similarly, estradiol is undetectable in stage A animals and then remains high throughout gestation.

This study suggests that the primary follicular steroid is estradiol until the final state of gestation, when progesterone is detected for the first time, and when testosterone production, previously about 10% of estradiol synthesis, is approximately equal to that of estradiol. Other studies (Tsang, unpublished) have shown that plasma

Table 1.--Stage of Gestation/Cycle

Steroid	A	B	C	D
P	N.D.	N.D.	N.D.	4888 ± 263
T	N.D.	233 ± 38	294 ± 41	2160 ± 923
E	N.D.	2332 ± 68	3805 ± 736	1626 ± 481

Basal progesterone (P), testosterone (T) and estradiol (E) levels (pg/250,000 granulosa cells) produced by ovarian follicles of Squalus acanthias taken throughout the cycle. N.D. - non-detectable.

progesterone is high during stages A, B and C of pregnancy, and falls sharply in stage D. Since follicles do not appear to synthesize progesterone in vitro until stage D, these data suggest that the source of the plasma P is likely to be the corpora lutea, which are known to have an active 3β HSD enzyme (Lance and Callard, Gen. Comp. Endo. 13:255-267, 1969). Although the functions of the gonadal steroids in elasmobranchs have not been fully elucidated (see Dodd, Dodd and Duggan in Control Processes in Fish Physiology, edited by Rankin, Pitcher and Duggan, pp 221-250, 1983), current work from this laboratory had implicated estradiol as a necessary adjunct for relaxin activity in the regulation of reproductive tract diameter in the "cervical" region (Koob, Laffan and Callard, unpublished). Supported by NSF PCM 8104144 to IPC.

GLOMERULAR HANDLING OF MACROMOLECULAR IRON DEXTRAN COMPLEXES BY THE KIDNEY OF THE ATLANTIC HAGFISH, Myxine glutionosa L.

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Myxine glutinosa is an osmoconformer, i.e., the plasma is isosmolar to the ambient seawater. Its kidneys consist of large paired glomeruli draining into two archinephric ducts via short neck segments (Stolte, H., Eisenbach, G.M., Bull. MDIBL 13:120-121, 1973).

The glomeruli show a pronounced mesangium extending into the peripheral capillary loop as a thick sub-endothelial layer between the endothelial cells and the basal lamina of epithelial cells. The glomerular capillary endothelium is remarkable for its few fenestrations (Kuehn, K., Reale, E., and Stolte, H., Cell Tissue Res. 164:201-213, 1975). In the present study, the mesangial access of macromolecules has been examined using iron dextran complexes (IDC) as a marker.

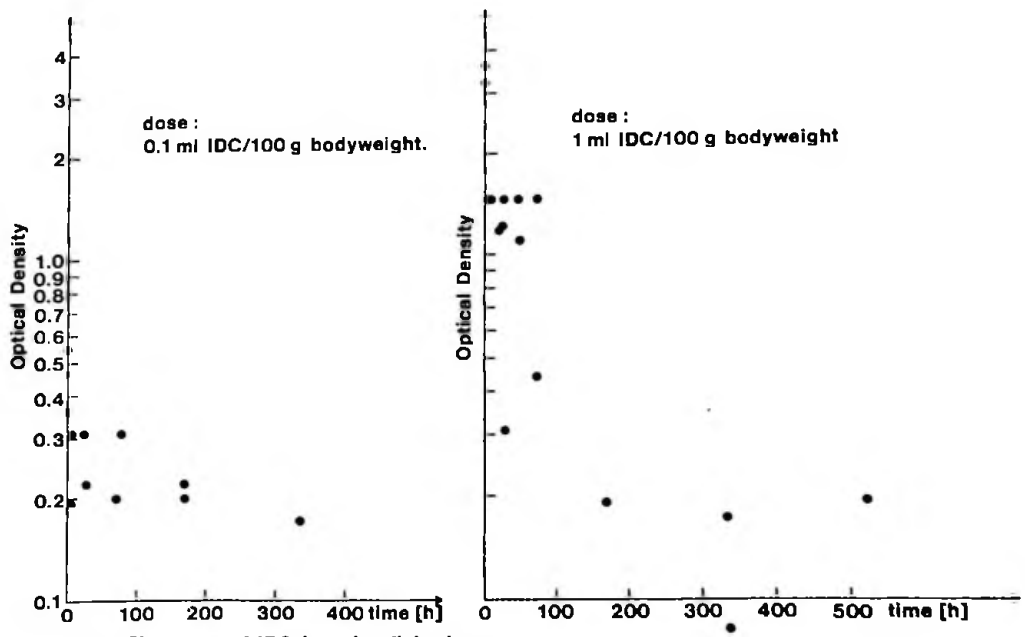

fig. 1 : Clearance of IDC from hagfish plasma
Each point represents one animal