It may be seen, that the flux per unit area of exposed tissue of $^{35}S\text{-}SO_4$ from serosa to mucosa under control conditions, and in the presence of both theophylline and SITS is similar in both species. The addition of theophylline produces a significant ($P < 0.005$) increase in flux, consistant with a raised mucosal border anion conductance (Naftalin and Simmons 1979, J. Physiol. 290:331-350). The reduction in basolateral border SO4 permeability induced by SITS (Smith,Orellana & Field, 1981, J. Membrane Biol. 63, 199), reverses this action of theophylline (ref. Naftalin & Smith, 1983).

The flounder and the rabbit appear to share a common response to the secretagogue theophylline. Following exposure to theophylline, they both exhibit an increased anion flux. Inhibition of this increased flux by SITS demonstrates that it has a transcellular component. Previously, it has been suggested that the failure of flounder intestine to show anion secretion is due to its lack of crypts, this is used as indirect evidence for the role of crypts in mammalian intestinal secretion (Field, Smith and Bolton [1980] 55:157). The data presented here shows that regulation of transcellular anion flux is present in flounder intestine and hence that the presence of crypts is not necessary to the regulation of anion secretion in either flounder or mammalian intestine. This work was supported by grants to R.J.N and P.M.S. from NATO and grants from the Royal Society and the Dale Fund to P.M.S.

THE RELATIONSHIP BETWEEN 2-D-DEOXY-D-GALACTOSE SECRETION AND ION COTRANSPORT ACROSS THE BASO-LATERAL BORDERS OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) INTESTINE

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The Serosa to Mucosa (s-m) flux of ^{86}Rb was measured across preparations of Flounder intestine stripped of serosa and outer muscle layers and mounted in modified Ussing chambers (Naftalin & Kleinzeller, Am. J. Physiol. 240:G392-G400, 1981). These measurements were correlated with transepithelial fluxes of ^{3}H labelled 2-deoxy-D-galactose (2-d-gal).

Flux values in nM $cm^{-2}Hr^{-1}$ were calculated from scintillation counts by conventional methods (Table 1).

Table 1.--S-M flux of ^{86}Rb nM cm^{-2} Hr^{-1}

		+furosemide (0.5 mM)	
Control	115.0 +/- 8.3 (27)	86.6 +/- 6.9 (15)	**
+Mannitol (10 mM)	203.3 +/- 28.1 (6)	125.6 +/- 38.1 (6)	*
+2-d-gal (10 mM)	172.1 +/- 26.9 (6)	83.2 +/- 13.4 (4)	***
low Na (10 mM)	102.8 +/- 15.0 (8)	95.9 +/- 13.3 (8)	
low Cl (10 mM)	78.5 +/- 13.4 (8)	83.2 +/- 9.2 (8)	

Low Na, Na replaced with choline, low Cl, Cl replaced with gluconate. +Indicates a comparison to control, *indicates comparison plus or minus furosemide. One symbol = $P < 0.05$, two symbols = $P < 0.02$, three symbols = $P < 0.01$.

^{86}Rb s-m flux into a bathing solution containing 1 mM K was reduced by 25% ($P < 0.01$) following the addition of furosemide to the serosal bathing solution. Replacement of Ringer Cl on the serosal side by gluconate led to a similar reduction of ^{86}Rb flux (32% $P < 0.01$). Addition of furosemide to low Cl Ringer did not elicit any further inhibition in ^{86}Rb glux.

While there appears to be a reduction in ^{86}Rb flux in low Na Ringer, the variation within these experiments does not permit any definite conclusions about the requirement for Na to be drawn from this data alone, however the furosemide-dependence of s-m ^{86}Rb flux is absent in low Na Ringer.

The sensitivity of the s-m flux of ^{86}Rb to furosemide indicates that ^{86}Rb crosses the epithelium via a Na-K-Cl

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