Table 2. The urine flow rates, urine to plasma ratios and clearance values of inulin, polyethylene glycol (PEG) and winter flounder antifreeze peptide translation product (WFAFPTP) in several species of fishes from the Gulf of Maine. Values expressed as the mean of n ± one standard error of the mean.

species	marker	injected	n[a]	fish weight (kg)	urine flow (ml/hr/kg)	urine/plasma ratio	clearance (ml/hr/kg)	clearance ratio[b]
Trematomus bernacchii[c]	inulin	i.v.	1(-)	0.20	-	0.06		
Myoxecephalus scorpius	inulin	i.v.	2(10)	0.56±.27	0.39±.04	0.93±0.22	0.36±.04	
Hemitripterus americanus	inulin	i.v.	2(16)	1.12±.05	0.14±.01	6.31±1.29	0.88±.12	
Macrozoarces americanus	PEG	i.v.	4(29)	0.45±.06	0.11±.01	3.34±0.61	0.37±.05	
	WFAFPTP	i.v.	2(5)	0.34±.02	0.21±.20	0.18±0.04	0.04±.04	0.11
Pseudopleuronectes americanus	PEG	i.v.	4(19)	0.44±.06	0.35±.08	1.94±0.48	0.68±.19	
	WFAFPTP	i.v.	1(5)	0.23	0.16	0.20	0.03	0.04
	WFAFPTP + PAN[d]	i.v.	1(7)	0.28	0.44	0.15	0.07	0.10
	WFAFPTP + PS[e]	i.v.	1(9)	0.28	0.32	0.25	0.08	0.12

[a] number of fish (number of clearance periods)

[b] clearance of WFAFPTP/clearance of PEG

[c] aglomerular (Dobbs et al., Science: 185, 793-794, 1974)

[d] puromycin aminonucleoside, 1 mg every 3 days for 12 days

[e] protamine sulfate, single injection of 2 mg

Although the results are preliminary, both puromycin aminonucleoside and protamine sulfate appear to enhance the clearance of the WFAFPTP. This increase in clearance of the antifreeze suggests that the mechanism of renal conservation results from a charge repulsion which occurs between the acidic peptide antifreeze and the polyanionic glomerular basement membrane. This research was supported by NSF Grant PCM 77-25166.

A THYROID NEOPLASM IN THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The reported incidence of spontaneous tumors and tumor-like lesions in elasmobranch fishes is very low. In a survey made in 1968, Wellings cited fourteen instances (Wellings, Nat. Cancer. Inst. Monog. 31, 59-128); that number has since been doubled (in the yearly Accession Lists, Registry of Tumors in Lower Animals, Smithsonian Institute), but still remains far below the incidence for bony fishes. Most of the recorded tumors of elasmobranchs are melanomas and external tumors, but five lesions of the thyroid gland have been listed. Cameron and Vincent (J. Med. Res. 27, 251-256, 1915) described a thyroid carcinoma in the dogfish, Squalus suckleyi, and a possible adenoma was found in a nurse shark, Ginglystoma cirratum (#RTLA 1836. Registry of Tumors). Recently, thyroid lesions have been noted in three Chondrichthyes from the Ueno Zoo Aquarium, Japan, including thyroid hyperplasia, colloid goitre and adematous goitre or follicular adenoma (RTLA 1851, 1852, 1853).

During August 1979, we dissected a large sexually mature female dogfish in the second year of gestation, weighing 7.2 kg which was carrying 15 young in utero. The thyroid gland was greatly enlarged and weighted 1.1 g; this is about 5-10 times the usual weight for the thyroid of fish of this size. The gland was symmetrically enlarged, translucent and flabby. There were no nodules, but the connective tissue sheath was thickened. The microscopic appearance of the gland was variable. Some areas, which tended to be at the edges of the gland, were composed of normal thyroid follicles, spherical in shape, bordered by low cuboidal epithelium, and filled with eosinophilic colloid. There was no conspicuous increase in vascularity or interfollicular stroma in these areas. In most of the gland, however, the thyroid follicles had become enormously distended with colloid and the diameter of these enlarged follicles was about 5-8 times normal suggesting an 80-fold increase in volume. The follicular epithelium was flattened and stretched and a few follicles showed intrafollicular haemorrhage. In some of the oversized follicles, the follicular epithelium had become hyperplastic and large papillae projected into the lumen (Fig. 1). Clusters of secondary follicles were seen in these papillae. There were large foci of stromal fibrosis throughout the gland.

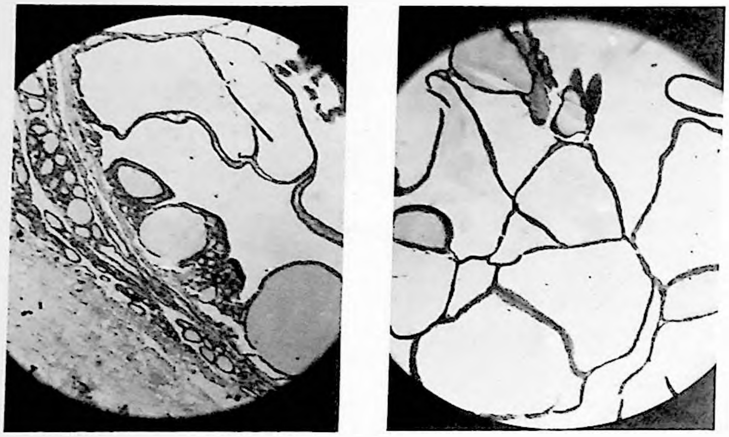

Figure 1. Sections through the thyroid gland showing (a) papillae with secondary follicles and focus of stromal fibrosis and (b) area of oversized follicles.

In mammals it is difficult to distinguish pathologically between adenomatous goitre and papillary adenocarcinoma. Features which are used to distinguish these lesions in mammals may be inappropriate for elasmobranchs. Nor can recourse be taken to the literature for bony fishes, where reports of thyroid tumors are far more numerous, but the situation confused. Indeed, Mawdesley-Thomas (in Pathology of Fishes, 805-807, 1975) has questioned the designation of 31 out of 35 such thyroid tumors, and has suggested that they were goitrous glands.

Hyperplastic goitrous thyroids result from a deficiency in the output of thyroid hormone in response to a lack of iodine in the environment, or from continuous exposure to goitrogens. Thus Sonstegard and Leatherland (Cancer Res. 36, 4467-4475, 1976) have suggested that thyroid hyperplasia in Coho Salmon living in Lake Ontario is caused by continuous exposure to environmental pollutants, in a freshwater environment, low in iodine. Such a situation is unlikely to be applicable to the migratory marine spiny dogfish for which iodine is not limited. We, therefore, suggest that the growth is a neoplasm.

There is a further, very speculative pointer in our diagnosis. Spontaneous hyperthyroidism in mammals usually may be associated with adenoma of the gland, whilst goitre is almost always associated with low circulating levels of thyroid hormones. The young carried in utero by this female had attained the same length as young carried by unaffected females, but weighed less and had notably less yolk remaining in their yolk-sacs (Table 1).

Table 1

A comparison of the intrauterine young carried by a female with a thyroid adenoma (A) and a normal female (B)

	# young	length (cm)	Total Wt. g	Body Wt. g	Yolk Wt. g
A	15	22.9 ± .15	*47 ± .29	*38 ± .51	*9.4 ± .43
B	13	22.9 ± .23	61 ± .73	45 ± .85	16.3 ± .56

*Indicates a significant difference at 1% level.

It is well known for higher vertebrates that the thyroid gland influences metabolic rate. If thyroid hormones similarly affect metabolism in elasmorbranchs and circulating levels were high in the mother as a result of the adenoma, the maintenance metabolism of the young may have been increased, and consequently more of the yolk reserves utilized. Since the mother does not contribute to the nutrition of the young during pregnancy, and the embryo is, in this respect, a "closed" system, any increased utilization for maintenance metabolism might well result in a smaller body weight, as less energy is available for growth.

DRAINAGE OF INTERSTITIAL FLUID FROM BRAIN IN THE LITTLE SKATE (RAJA ERINACEA)

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The classical view of the drainage of interstitial fluid (ISF) from brain is of a flow of perineuronal fluid into a canalicular system of perivascular spaces with eventual drainage into cerebrospinal fluid (CSF). Recent experiments in rat and rabbit have confirmed this view; they also indicate additional pathways of ISF drainage from brain, namely into blood vessels in the choroid plexus, along the optic and olfactory nerves, and into the deep cervical lymphatics (Cserr et al. Exp. Eye Res., Suppl. Vol. 25:461-473, 1977; Cserr & Bradbury, In Preparation). In order to investigate these extra-CSF pathways of fluid removal from brain we have studied the flow of cerebral ISF in the little skate, Raja erinacea. In this species the ventricular cavities are extremely reduced and there is no subarachnoid space.

Three test compounds were employed as markers of ISF flow: albumin complexed to Evans Blue (EBA), horse-radish peroxidase (HRP), and radioiodinated serum albumin (RISA). Balanced saline solution containing one of the test compounds was injected slowly into the telencephalon ($< 0.3\ \mu l$/min) through a 30 gauge cannula attached via teflon tubing to a Harvard infusion pump. Channels of flow were then identified as the pathways of distribution away from the injection site.

In initial experiments with EBA, 2 to 14 μl of dye solution (2.5 protein; 5% dye) was injected into brain and the subsequent appearance and distribution on the dorsal surface of the brain observed for 1 to 3 hours through a dissecting microscope (N=5). The pathways of flow outlined by the blue dye indicated that flow follows the course of blood vessels. The marker could not be followed for more than 1 to 2 cm from the injection cannula, however, presumably because of dilution of the test compound.

In order to investigate the pathways of flow and drainage with greater resolution and sensitivity we used the protein tracer HRP. One μl of a 30% solution of Sigma's type VI HRP was injected into the telencephalon. The skates were sacrificed either 15 min (N=4), 4 hr (N=4) or 24 hr (N=4) after injection and the brain fixed by vascular perfusion with a mixed aldehyde fixative. The fixed tissues were reacted for peroxidatic activity and processed for light or electron microscopy. Preliminary examination at the light level confirms the role of perivascular spaces as preferential channels for the flow of ISF. Also in agreement with previous work, the histological distribution of HRP in the skate indicates flow of ISF to the subependymal layer and between ependymal cells to the ventricular lumen. HRP was also seen in the lumen of some superficial veins associated with the meninges. These observations will be extended to the electron microscopic level.

The elasmobranch brain is surrounded by extradural fluid (EDF). Results with the three protein tracers indicate exchange between this fluid and the brain in the little skate. HRP and RISA distributed to EDF following intracerebral injection and EBA stained perivascular spaces surrounding the superficial meningeal vessels following injection into EDF. These results are at variance with the generally accepted view that EDF is isolated from the brain by a brain-EDF barrier. This work was supported by U.S. Public Health Service Grants NS11050 and NS13844.