are considerably higher than those of humans. In *Hylobates lar,* the total serum protein concentration is lower than in humans, while the protein-bound hexosamine level is very close to human levels. The electrophoretic patterns of the four species of nonhuman primates examined are very different from that of humans and from each other.

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Inhibition of Water Diuresis in *Mustelus canis* by Extracts of Homologous Brain and Spinal Cord* (32859)

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Marine elasmobranchs maintain their body fluids hyperosmotic to sea water, continually taking up water through their gills and integument, while maintaining water balance through the renal excretion of a urine which is hypo-osmotic to both sea water and plasma. An increased uptake of water occurs when these fish migrate to fresh water or are placed in dilute sea water. In these circumstances plasma osmolality becomes reduced, urine flow rate and glomerular filtration rate increase, and the ratio of urine osmolality to plasma osmolality decreases (1). A similar response to dilution is observed in higher vertebrates (2–4) and it has been repeatedly suggested that endocrine control of water excretion exists in the elasmobranchs as it does in higher forms. In teleosts the caudal neurosecretory system of the spinal cord has been implicated in the control of water excretion, and the neurosecretory cells in the spinal cords of elasmobranchs have been considered as having analogous functions (5,6). However, despite many conjectures no experimental evidence has supported the presence of a humoral factor capable of influencing water excretion in elasmobranchs, nor is there evidence directly implicating the caudal neurosecretory cells or any other elasmobranch tissue as the source of these postulated factors. In a search for such factors we have examined the effect of injections of extracts of various tissues taken from the smooth dogfish, *Mustelus canis,* on the renal function of other *Mustelus* undergoing water diuresis while in dilute sea water.

Methods. Donor fish, (male and female, weighing between 1 and 4 kg) were placed in fresh running dilute sea water (70–80%) for 2–3 days, a period sufficient to promote development of the maximum drop in plasma osmolality and the maximum water diuresis obtainable in this salinity. Donor fish were then anesthetized with intravenous sodium pentobarbital. Tissues were removed and frozen. Four to 5 gm of tissue were homogenized in the cold, mixed with an equal volume of dogfish Ringer solution (7), and this mixture was centrifuged at 500 or 12,000*g*. The resulting supernatant fluid was frozen until injected into the caudal vessel of a recipient fish.

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TABLE I. Effect of Injection of Spinal Cord and Brain on Urine Flow Rate, Glomerular Filtration Rate, Urine/Plasma Osmolality Ratios and Plasma Osmolality of *Mustelus canis*. (Average, range, number of collection periods, and number of fish)

	Dilute sea water		
	Preinjection periods	Postinjection period	Sea water
Urine flow rate (ml/hour per kg)	1.51 (0.70–2.16) 19 periods, 7 fish	0.47 (0.39–0.55) 5 periods, 5 fish	0.58 (0.22–0.88) 24 periods, 3 fish
Glomerular filtration rate (ml/hour per kg)	3.32 (2.18–5.00) 12 periods, 7 fish	1.08 (0.69–1.88) 5 periods, 5 fish	[a]
Urine/plasma osmolality	0.78 (0.70–0.88) 19 periods, 7 fish	0.89 (0.85–0.97) 5 periods, 5 fish	[a]
Plasma osmolality (milliosmols/kg of H_2O)	803 (743–866) 12 periods, 7 fish	800 (787–842) 5 periods, 5 fish	[a]

[a] Not measured in present experiment. Kempton (11) reports glomerular filtration rate in *Mustelus canis* in sea water to be 1.82 ml/ hour per kg.

Recipient fish (females only, weighing between 4 and 8 kg) were kept in dilute sea water (70–80%) throughout an entire experiment and were injected with inulin and catheterized for the collection of urine after 48 hours in this environment. Procedures used for the collection of urine and blood, and the determination of osmolality and glomerular filtration rate (inulin clearance) have been previously described (8,9). Injection of tissue extracts was preceded by 2–3 collection periods covering 24–36 hours in all, and was followed by one collection period of 2 hours and one or more subsequent collection periods of 4–24 hours.

Results. Table I presents data obtained from recipient fish prior to injection. Data on urine flow in fish maintained in sea water but otherwise treated similarly to fish in dilute sea water are also included. Since considerable variation in absolute values of urine flow rate, glomerular filtration rate, and urine/plasma osmolality ratios occurred from period to period and from fish to fish during preinjection control periods, the effect of administration of extracts is most readily identified when the percentage change between two consecutive collection periods is examined (Table II). The changes in urine flow, urine/plasma osmolality ratio, and glomerular filtration which occurred between consecutive periods prior to injection of an extract varied in magnitude and direction. Injection of 4 ml of dogfish Ringer solution (into 1 fish) or 4 ml of extracts of parietal muscle (into 1 fish) or of liver (into 2 fish) had no effect on urine flow, urine/plasma osmolality ratio or glomerular filtration rate when changes between the period before injection and the period after injection were compared with changes between two preinjection periods (Table II). The injection of 4 ml of extracts of the caudal two-thirds of spinal cords (into 3 fish) or 4 ml of extracts of brain (into 2 fish) always resulted in a large drop in urine flow rate and glomerular filtration rate and a rise in the urine/plasma osmolality ratio. These changes were considerably larger and were significantly different from those changes seen in preinjection periods (Table II). No significant change in plasma osmolality occurred after any injections. Data obtained in the 2-hour period following injection of spinal cord or brain extracts are shown in Table I. The mean urine flow rate during this period was within the range seen in nondiuretic fish. In subsequent collection periods lasting from 4 to 24 hours, urine flow rate, glomerular fil-

TABLE II. Urine Flow Rate, Glomerular Filtration Rate, Urine/Plasma Osmolality: Percentage Change Between Two Consecutive Periods. (Mean; range; number of paired collection periods; significance of difference from values in preinjection control periods, Student's t test.)

	Preinjection dilute sea water	Injection of liver, muscle, or Ringers	Injection of spinal cord or brain
Urine flow rate	−1.5% −37–+38% (12)	−2.6% −14.5–+38% (4) NS	−67.6% −79–−59% (5) $p < .001$
Glomerular filtration rate	+0.9% −26–+38% (4)	+12.2% −0.9–+71% (4) NS	−70% −80–−55% (5) $p < .005$
Urine/plasma osmolality	−1.36% −18.2–+16.4% (11)	−1.82% −10.8–+5.01% (4) NS	+12.7% +4.85–+37.3% (5) $p < .05$

tration rate, and urine/plasma osmolality ratio either remained altered or tended to return to preinjection values.

Discussion. Apparently extracts of brain and spinal cord, but not extracts of liver or muscle, contained factors which reversed the diuresis produced by dilution. This reversal was primarily dependent upon a reduction of the glomerular filtration rate towards non-diuretic levels. In all likelihood this reduction of glomerular filtration rate was brought about by constriction of the renal vasculature. After injection of extracts of spinal cord or brain we observed the tail blanch and noticed that blood drawn 2 hours after injection was considerably darker than when otherwise collected. These observations suggest that vasoconstriction in skin vessels and gill vessels also occurred.

The increase in the urine/plasma osmolality ratio suggests that factors capable of increasing the permeability of the nephron to water were also present, although large decreases in tubular flow rate secondary to reduced glomerular filtration rate may have facilitated nephron water reabsorption or, perhaps, decreases in solute reabsorption in water impermeable nephron segments may also have influenced this change.

These results provide no information concerning the composition of the antidiuretic factors in extracts of brain or spinal cord nor do they indicate whether these factors play a physiological role in controlling water excretion in these fish. Injection of vasopressin, oxytocin, or extracts of homologous pituitary glands into the spiny dogfish, *Squalus acanthias,* has no effect on a water diuresis produced by immersion of these fish in 75% sea water (B. Truniger, B. Schmidt-Nielsen, L. Rabinowitz, unpublished observations). Epinephrine, injected into *Squalus acanthias,* produces a solute diuresis (10). Consequently these substances are probably not the antidiuretic factors found in the present experiment. Since neurosecretory cells have not been reported in brains of elasmobranchs, it remains unclear whether these cells are involved in the antidiuresis produced in these experiments.

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The Passage of Oxygen through Isolated Sheets of Human Stratum Corneum* (32860)

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Since the oxygen content of the skin, and deeper tissues, of the human limb is dependent, in part, upon that of the external environment (1–3), since enough oxygen may come from this route to benefit an ischemic limb, and since the factors that play a role in this process still require further investigation (4–6), we have studied one of these, the passage of oxygen through the stratum corneum. These experiments were prompted by the fact that intact sheets of human stratum corneum can now readily be obtained (7).

We have measured the rate of passage of oxygen when such sheets are placed between air and an essentially oxygen-free liquid because this simulates, to a large extent, those conditions under which the very ischemic limb exists (2).

Method. The method of preparing the sheets of stratum corneum from the abdominal wall of human cadavers, and the chamber used to measure the passage of oxygen through these sheets, have been described elsewhere (7,8).

The phlanges of both the upper and lower sections of the chamber were first thoroughly cleaned and coated with a thin film of Fisher Cello-Seal. A sheet of stratum corneum was placed between the two sections; care was taken to make sure that the central portion (12-mm diameter) of the membrane through which the oxygen would pass was not touched. Then the sections were clamped together; pressure was applied evenly to avoid tearing of the sheet. A magnetic stirring bar was inserted into the lower section of the chamber *via* its sidearm, and nitrogenated (deionized) water was then quickly injected through this sidearm. A Beckman-Clark oxygen electrode (model 160) was then inserted into the water, just long enough to obtain an oxygen tension reading. Finally, the rim of the sidearm, previously coated with Cello-Seal grease, was sealed with aluminum (Al-foil).

The upper section of the chamber then was filled approximately halfway with 4 ml of (deionized) water which had been equilibrated with air; the upper surface of the water was left uncovered, in contact with the outside air. (The volume of the lower section is 10 ml, including the sidearm.)

The chamber was submerged into a water bath of 37°C up to the level of the phlange joint, and the actual experiment was begun by activation of the magnetic stirrer. It was terminated 4 hours later, after which time the Al-foil was removed from the sidearm and another oxygen reading of the water taken. The increase in reading over the original value was calculated, per minute, per micron thickness of the stratum corneum.

Materials of known oxygen permeabilities were similarly studied, as controls, in a number of experiments. These consisted of a material of essentially no permeability (Al-foil), of two very permeable materials (Teflon, 12-μ thick, and cellulose acetate, 22–μ thick), and one less permeable (Kodak Kodar, Mylar type, 19-μ thick).

Results. See Table I. The amount of oxygen that passed through the stratum corneum did

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