

Figure 1

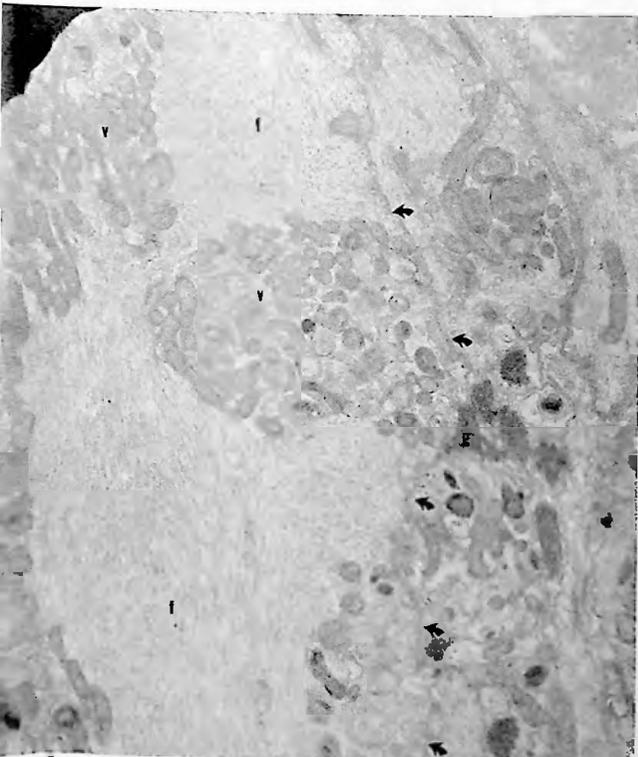


Figure 2

...etermine whether these factors are of general im-  
 ...ance in the mechanism of central nervous system  
 ...eneration in lower vertebrates.

Hagfish spinal cord resembles lamprey spinal cord in  
 ...ny respects. It is a thin dorso-ventrally flattened

structure. The gray matter is reduced and there are no recognizable dorsal horns. A few large neurons are present laterally. Hagfish neurons are distinguished from other vertebrate neurons primarily in the frequent occurrence of dense and crystalline material within their perikarya. The white matter contains axons of various sizes, although none reach the diameters of the giant axons in lamprey cord. In other respects, hagfish axons resemble lamprey axons. Large amounts of fibrillar glial cytoplasm seems to be associated with most or all axons. Unlike the larval lamprey, capillaries are present within the spinal cord of hagfish.

The spinal cord was transected in 9 hagfish. The animals were anesthetized with Tricaine and perfused through the mouth and gills with sea water. A midline ventral incision in the midthoracic region exposed the vertebral column. The cartilage was removed and the spinal cord transected. The muscle was then sutured and the cut edges of the skin were approximated with wound clips. Seven hagfish survived. These animals were killed from 4 to 21 days postoperatively by decapitation or by intracardiac perfusion with Karnovsky's fixative.

The site of the transection was marked by an intense vascular reaction. The density of capillaries within the spinal cord was apparent.

At 4 and 7 days postoperatively, degenerating boutons and axons could be seen in the spinal cord both rostral and caudal to the lesion. Synaptic vesicles were often swollen. At 14 and 21 days, chromatolytic neurons could be identified in the gray matter. Large axons rostral to the transection commonly contained accumulations of vesicular material dense-cored vesicles and mitochondria. The axonal contents were often compartmentalized, usually by smooth endoplasmic reticulum membrane (Figure 1). At 21 days large axons contained densely packed neurofibrils with peripheral accumulations of mitochondria and vesicular material. These axons, which were presumably regenerating, were surrounded by fibrillar glial cytoplasm (Figure 2).

Preliminary evidence therefore suggests that hagfish spinal neurons are capable of regeneration and that this process may be characterized by both a neuronal synthetic reaction (chromatolysis) and by close glial association with the regenerating axon.

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#### Renal and Endocrine Response of the Dogfish to Change in Sea Water Concentration

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Homer Smith stressed the role of urea in osmoregulatory adaptation by elasmobranchs (*Biol. Rev.* 11:49, 1936). Forster further summarized these osmoregulatory

mechanisms of elasmobranchs emphasizing the role of non-electrolyte solute, urea, and the solutes sodium and chloride (*Sharks, Skates and Rays, 1967, pp. 187*). In our own studies of urea and sodium excretion in the dogfish, *Squalus acanthias*, we found parallel changes in urea and sodium reabsorption by the kidney regardless of the challenges used, diuretics, epinephrine, or phloretin (*Bull. MDIBL 14:77, 1974*).

With diuretics there was not a consistent response found in potassium excretion when natriuresis was induced (*MDIBL 13:88, 1973*). In initial studies of the renal response of the dogfish to dilute water environment it was noted that potassium excretion decreased when natriuresis ensued. These findings led to the current studies designed not only to quantitate solute changes in response to sea water salinity changes, but to evaluate potential humoral regulatory mechanisms involved.

Six female dogfish were studied using renal clearance techniques previously described (*Bull. MDIBL 11:71, 1971*). Inulin, as a 31/3% solution in dogfish Ringers, was administered intravascularly 12 to 18 hours before each series of renal clearance studies.

Each dogfish was studied the first day in full strength sea water, then placed in approximately 70% sea water for three days, then returned to full strength sea water. Renal clearance studies were repeated each day in the dilute sea water and after return to full strength sea water.

Each day of study timed urine collections were accomplished and appropriate blood samples were obtained for clearance studies. In addition, blood samples were obtained and plasma rapidly separated and frozen for steroid determinations. Blood and urine collections

obtained for clearance studies were analyzed for inulin, urea, sodium, potassium, calcium and magnesium concentrations.

The concentration of sea water was monitored frequently with a calibrated and sensitive hygrometer. Random determinations of osmolality were used to verify the validity of the hygrometer monitor.

Results of the clearance studies are given in Table.

After the dogfish were placed in the dilute sea water there occurred in each a brisk diuresis and an increase in inulin clearance (G.F.R.) that persisted throughout the stay in dilute sea water. An increased fraction of filtered volume was excreted. Sodium and urea excretion increased 3 fold and 10 fold respectively, with increased fraction of filtered sodium and urea excreted. Plasma sodium concentration decreased to a new steady concentration by the second day. Plasma urea concentration decreased proportionally more than the decrease in plasma sodium concentration.

Plasma potassium concentration decreased the first day in dilute sea water, then gradually returned to control values. There occurred a decrease in potassium excretion the first day, with potassium excretion increasing as the fish stayed in the dilute environment. The high variability in potassium excretion during the control period in different fish made the change noted in potassium excretion during the control period in different fish meaningless. The change noted in potassium excretion not statistically significant. The ratio of potassium excreted to potassium filtered did decrease significantly during the first two days in the dilute sea water. With the prominent increase in filtered potassium load there occurred relative potassium

Table I

(Values with time as a function are expressed as unit/Kg/hr and given as mean  $\pm$  standard deviation)

	Control in 100% Sea H <sub>2</sub> O	Day 1	~ Sea H <sub>2</sub> O Day 2	Day 3	Return to 100% Sea H <sub>2</sub> O
Volume, ml	0.60 $\pm$ 0.24	2.87 $\pm$ 0.64*	3.38 $\pm$ 0.98*	2.87 $\pm$ 0.87	0.47 $\pm$ 0.19†
C <sub>in</sub> , ml	2.77 $\pm$ 0.82	5.28 $\pm$ 1.59*	5.99 $\pm$ 1.59*	5.22 $\pm$ 1.49*	1.55 $\pm$ 0.35†
P <sub>urea</sub> , mM/L	356 $\pm$ 15	306 $\pm$ 24*	280 $\pm$ 15*	264 $\pm$ 11*	283 $\pm$ 21*
U <sub>urea</sub> V, $\mu$ M	46 $\pm$ 24	485 $\pm$ 146*	555 $\pm$ 198*	401 $\pm$ 127*	42 $\pm$ 22†
U <sub>urea</sub> V/F <sub>urea</sub>	0.048 $\pm$ 0.020	0.305 $\pm$ 0.082	0.031 $\pm$ 0.072*	0.031 $\pm$ 0.081*	0.096 $\pm$ 0.042†
P <sub>Na</sub> , mEq/L	264 $\pm$ 6	237 $\pm$ 4*	229 $\pm$ 3*	228 $\pm$ 4*	251 $\pm$ 8*†
U <sub>Na</sub> V, $\mu$ Eq	153 $\pm$ 56	468 $\pm$ 101*	512 $\pm$ 174*	392 $\pm$ 139*	66 $\pm$ 42†
U <sub>Na</sub> V/F <sub>Na</sub>	0.209 $\pm$ 0.034	0.379 $\pm$ 0.056	0.367 $\pm$ 0.048*	0.327 $\pm$ 0.061*	0.161 $\pm$ 0.086†
P <sub>K</sub> , mEq/L	3.34 $\pm$ 0.15	3.16 $\pm$ 0.16*	3.29 $\pm$ 0.19	3.32 $\pm$ 0.19	3.80 $\pm$ 0.21*†
U <sub>K</sub> V, $\mu$ Eq	23.3 $\pm$ 14.5	12.3 $\pm$ 8.8	23.8 $\pm$ 14.3	35.6 $\pm$ 24.2	32.7 $\pm$ 22.2
U <sub>K</sub> V/F <sub>K</sub>	2.77 $\pm$ 1.92	0.80 $\pm$ 0.57	1.30 $\pm$ 0.92*	2.21 $\pm$ 1.53	5.54 $\pm$ 3.53†
P <sub>Ca</sub> , mEq/L	4.36 $\pm$ 0.50	3.65 $\pm$ 0.39*	3.46 $\pm$ 0.29*	3.17 $\pm$ 0.20*	3.55 $\pm$ 0.44*
U <sub>Ca</sub> V, $\mu$ Eq	6.6 $\pm$ 3.5	11.0 $\pm$ 1.9*	10.8 $\pm$ 3.1*	7.4 $\pm$ 2.6	2.3 $\pm$ 0.9†
P <sub>Mg</sub> , mEq/L	157 $\pm$ 0.28	1.21 $\pm$ 0.24*	1.02 $\pm$ 0.13*	0.98 $\pm$ 0.11	1.50 $\pm$ 0.28†
U <sub>Mg</sub> V, $\mu$ Eq	15.6 $\pm$ 61	14.0 $\pm$ 3.2	14.3 $\pm$ 6.5	13.5 $\pm$ 5.3	12.0 $\pm$ 5.3

\* p<.01 versus control

† p<.01 versus 70% sea H<sub>2</sub>O

Response of *Squalus acanthias* to Angiotensins.

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Elasmobranchs are not known to produce renin since they lack a renal juxtaglomerular apparatus (Nishimura et al., *Am. J. Physiol.* 218:911-195, 1970). However, *Squalus acanthias* does exhibit a strong and equipotent pressor response to both Angiotensin I (AI) and Angiotensin II (AII) the peptides which result from the action of renin on a plasma protein substrate. A specific inhibitor of the enzyme which converts the decapeptide Angiotensin I to Angiotensin II in higher species is available. It was of interest to ascertain whether *Squalus acanthias*, a representative of the Class Elasmobranchii, possesses the enzyme which converts AI to AII although the peptide substrate, Angiotensin I, is presumably absent in this species.

Unanesthetized female dogfish were restrained in a narrow shallow box provided with a continuous flow of cold sea water. Phasic dorsal aortic pressure and heart rate were recorded via a PE60 polyethylene catheter attached to a P23AA Statham gage and recorded by an Electronics-for-Medicine recorder. Respiratory movements were also monitored with a sea water filled PE260 catheter inserted into the pharyngeal cavity and attached to a similar recording system. Injections of AI, AII and the nonpeptide converting enzyme inhibitor SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-) were made via the dorsal aortic catheter according to the protocol shown in Table I. All compounds were made up to 1 ml injection volume and flushed in with 1 ml elasmobranch saline. Ten minutes intervened between control injections of AI and AII. AI or AII was injected 5 minutes after the administration of 3 mgm of SQ 20881 and 2 minutes after a subsequent injection of 0.3 mg of the inhibitor. The data were analyzed for significance by a paired "t" test program run on a Wang 600 calculator.

Table I presents the essential data. The maximum response to either AI or AII occurred within two minutes after injection without a significant difference in peak response time.

These data strongly suggest that *Squalus acanthias* may possess a dipeptidylcarboxypeptidase similar to, or identical with, that which converts AI to AII in higher forms. If so, it adds to the complexity of peptide physiology and its evolution. However, there may be an alternative explanation. Peach (*Circ. Res.* 28 (Suppl. II):11-107-117, 1971) found that in cats both AI and AII stimulated adrenal medullary catecholamine release. Dogfish are abundantly endowed with chromaffin tissue. In three experiments we found that an alpha adrenergic blocking agent (phentolamine) abolished the pressor effect of both AI and AII. A ganglionic blocking agent (hexamethonium) did not affect the pressor action of AII. From these observations, it may be hypothesized that in the dogfish the pressor effect of either AI or AII is brought about by direct stimulation of receptors in chromaffin tissue which release a catecholamine, presumably norepinephrine and/or epinephrine, whose

conservation by the nephrons. The initial decrease in plasma potassium may have been due to movement of potassium into the intracellular space.

Plasma calcium concentration was reduced throughout the stay in dilute sea water. This was associated with an initial increase in calcium excretion. There was no measurable change in fraction of filtered calcium excreted, with the increase in calcium excretion being thus parallel to the increase in filtered calcium load.

Plasma magnesium concentration also decreased progressively during the stay in dilute sea water, but unlike calcium, there was no measurable change in magnesium excretion. There was, however, a decrease in the ratio of magnesium excreted to magnesium filtered that persisted throughout the stay in dilute sea water.

Upon return to full strength sea water, urine production decreased to control values, with decreases in F.R. and fraction of filtered volume excreted.

Urine sodium and urea excretion also decreased to as low as control rates of excretion. Fractions of filtered sodium load and filtered urea load excreted also decreased toward control values. Plasma sodium and urea concentrations increased upon return to full strength sea water.

Plasma potassium concentration increased to above control values even though there occurred an increase in the ratio of potassium excreted to potassium filtered. These findings suggest that the prompt increase in plasma potassium concentration may have been due to movement of potassium from the intracellular space rather than resulting from a renal mechanism.

Magnesium changes after return to full strength sea water were similar to those found for potassium. Plasma magnesium concentration promptly increased to control values even though the ratio of magnesium excreted to magnesium filtered increased. The similarity to the change in plasma potassium are interesting since magnesium and potassium are both important intracellular cations.

Plasma calcium concentrations also increased upon return to full strength sea water, associated with increased urine calcium excretion.

The pattern of renal response to exposure to altered sea water salinity suggests a humoral mechanism such as mineralocorticoid. This is especially interesting since Churchill and co-workers (*Bull. MDIBL, report #1 this year*) did not demonstrate a decrease in sodium excretion in the dogfish in response to desoxycosterone acetate.

Aldosterone assay failed to demonstrate aldosterone in any of 28 plasma samples or in urines studied. This is in agreement with the findings reported by Truscott and Herter (*J. Endocrinol.* 40:515, 1968). Testosterone concentrations, determined because of known high levels of testosterone in elasmobranchs, did not show changes when dogfish were moved from full strength to 70% sea water, or after return to full strength sea water. Blood and urine samples from the studies and interrenal glands from normal dogfish are being assayed for other steroids that may have a mineralocorticoid effect.