onservation by the nephrons The initial decrease in lasma potassium may have been due to movement of otassium into the intracellular space.

Plasma calcium concentration was reduced throughut the stay in dilute sea water. This was associated with n initial increase in calcium excretion. There was no easurable change in fraction of filtered calcium xcreted, with the increase in calcium excretion being us parallel to the increase in filtered calcium load.

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

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

Urine sodium and urea excretion also decreased to as w as control rates of excretion. Factions of filtered dium load and filtered urea load excreted also creased toward control values. Plasma sodium and ea concentrations increased upon return to full strength a water.

Plasma potassium concentration increased to above ntrol values even though there occurred an increase in e ratio of potassium excreted to potassium filtered. ese findings suggest that the prompt increase in asma potassium concentration may have been due to ovement of potassium from the intracellular space ther than resulting from a rental mechanism.

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

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

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

Aldosterone assay failed to demonstrate aldosterone any of 28 plasma samples or in urines studied. This is in reement with the findings reported by Truscott and er (*J. Endocrinal. 40:515, 1968*). Testosterone conntrations, determined because of known high levels of smobranchs, did not show changes when dogfish were ved from full strength to 70% sea water, or after urn to full strength sea water. Blood and urine samples m the studies and interrenal glands from normal gfish are being assayed for other steroids that may ve a mineralocorticoid effect.

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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):II-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

Table I

Maximum Response to Angiotensins I and II Before and After Converting Enzyme Inhibitor (SQ 20881)

TREATMENT	Pressure Systolic	Pressure Diastolic	Heart Rate/min	Resp. Rate/min
1. a) Control	23.7±2.1	17.8±1.4	33.0±2.6	40.1±1.0
b) 20 μg A_I	31.9±2.6	24.6±1.4**	33.3±2.3	40.3±1.2
2. a) Control	22.7±2.0	17.8±1.5	34.0±2.3	36.7±3.8
b) 20 μg A_{II}	33.7±2.4**	26.2±1.4**	35.6±2.3	40.7±1.3
3. a) Control	24.0±1.6	18.3±1.1	33.1±2.4	41.3±1.2
b) After SQ 20881 (3.3 mg total)	23.8±1.5	18.9±1.1	33.3±2.7	40.6±1.1
4. a) Control (After SQ 20881)	22.4±1.2	17.9±0.9	33.9±2.7	41.0±1.3
b) 20 μg A_I	23.2±1.4	18.9±0.9	33.7±2.6	41.4±1.2
5. a) Control (After SQ 20881)	22.1±1.5	17.4±1.1	34.1±2.5	41.3±1.2
b) 20 μg A_{II}	34.3±2.6**	26.4±1.9**	34.6±2.3	41.4±1.4

N=10 ** P<.001

pressor action can be blocked at vascular receptor sites by an alpha adrenergic blocking agent. This research was supported by the Monmouth County Chapter, New Jersey Heart Association Affiliate.

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Solubilization, Separation and Partial Purification of Cytochrome P-450 and Cytochrome b_5 from Hepatic Microsomes of the Little Skate, *Raja erinacea*

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Investigations in our laboratory during the past few summers have shown that the little skate, *Raja erinacea*, has a hepatic drug-metabolizing system which is similar in many ways to analogous mammalian systems (*Bull. MDIBL 12*: 12, 1972; *13*:9, 1973; *14*:7, 1974). Thus, the little skate is capable of modifying lipophilic xenobiotics to more polar metabolites by a process known as mixed-function oxidation. The hydroxylation reactions carried out are catalyzed by cytochrome P-450, the terminal oxidase of the system. Our present understanding of mammalian mixed-function oxidations has been greatly increased by progress in the solubilization and purification of the membrane-bound components of the system and the reconstitution of hydroxylase activity from the purified components (see review, *Biochem. Biophys. Act 344*: 205, 1974). We have initiated similar studies on the hepatic drug-metabolizing system of the little skate in order to identify the components of and study the mechanisms of mixed-function oxidation in a marine species. Our progress in solubilizing and partially purifying cytochrome P-450 and cytochrome b_5 from hepatic microsomes of the little skate is reported here.

Little skates were caught locally and stored in live cars for a maximum of three days before use. Microsomes were prepared from livers (average w weight 24.3 g) as previously described (*Bull. MDI 12*:12, 1972) and suspended in 0.25 M sucrose for stora under nitrogen at -5 to -10°C. The yield of protein these preparations was 13.8 mg per g tissue; about 15 of microsomes was obtained in all. The samples we transported to NIEHS packed in dry ice and stored -20°C until used.

For solubilization, thawed microsomes (about 20 mg protein in 80 ml) were suspended to a final volume 200 ml in 100mM phosphate buffer, pH 7.7, contain 20% glycerol, 1 mM dithiothreitol (DTT) and 1 m EDTA. Sodium cholate was then added to a fi concentration of 1 mg detergent per mg protein and t suspension was stirred for 30 min at 4°C. The suspens was then centrifuged at 176,000g for 2 hr to precipita the undigested material. The clear, yellow supernata fraction was diluted 2-fold with a 20% glycerol solu containing 1 mM DTT and 1 mM EDTA and applied to column of DEAE-cellulose (2.5 x 45 cm) previous equilibrated with 50 mM phosphate buffer, pH 7. containing 1 mM DTT, 1 mM EDTA, and 0.5% chola (buffer I). Cytochrome P-450 was eluted from the colum with buffer I containing 0.3% Emulgen 913, a nonion detergent.

Fractions containing cytochrome P-450, obtained b chromatography on DEAE-cellulose, were combine diluted 2-fold with a 20% glycerol solution and applied a column of hydroxylapatite (2.5 x 6 cm) either directly o following concentration by ultrafiltration (Amicon, PI 100 membrane). The hydroxylapatite was equilibrate with 25 mM phosphate buffer, pH 7.7, containing 0.5 m DTT, 0.5 mM EDTA, and 0.25% cholate (buffer II). Afte application of the sample, the column was washed wit 50-100 ml of buffer II and a protein-containing fracti (fraction I) obtained. The cytochrome P-450 (fraction I was then eluted by increasing the buffer concentration t 140 mM and adding Emulgen 913 to 0.2%.