Table III

Thickness of the Cornea Measured with the Specular Microscope for an Elasmobranch and a Teleost and the Effect of Low Temperature

	Control				Low Temperature*		
Spiny Dogfish (*Squalus acanthias*)	Total Thickness Central	Total Thickness Periferal	Epithelium	Depth of Anterior Chamber	Total Thickness Central	Total Thickness Periferal	Epithelium
Means	161	184	66	342	159	155	67
±SEM	9	17	6	102	11	13	3
N	14	9	6	6	7	7	4

Longhorn Sculpin (*Myoxcephalus Octodecimspinous*)	Epithelium	Intermediate Layer of Stroma	Descemet-Enoothelium	Total Thickness of Cornea Control	Total Thickness of Cornea Cold *	Depth of Anterior Chamber
Means	14	124	213	241	820	725
±SEM	3	7	16	12	90	129
N	4	7	4	7	7	6

* Eye kept overnight a 2° C covered with Ringer.

In the teleosts the images obtained were 5. They correspond to the layers of relexion of the images in the complex cornea of these fish. The first two images corresponded to the anterior and posterior portion of the epithelium, the third to the anterior or dermal portion of the stroma, and the fourth and fifth to the outer and inner limits of the Descemetendothelial zone. The actual thickness of the regions and the effect of low temperature is shown in Table 3. It is evident that the cornea of teleosts swells and increases its thickness in the cold.

In summary, the ancient cornea of the shark, possesses the ability to remain at normal hydration and thickness in adverse metabolic conditions, and at the same time, no significant electrical potential difference is found arising from the epithelial layers. On the other hand, in the more recent teleosts, the cornea swells in the cold and shows he consistent presence of an electrical potential difference arising from the epithelium, similar to amphibians and mammalians. Specular microscope measurements of corneal thickness *in vivo* and in mounted corneas of sharks and sculpins is reported.

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Plasma And Urina Proteins In The Aglomerular Teleost, *Lophius americanus*

R. Galaske, State University of N.Y. at Buffalo and Medizinische Hochschule, Hannover

Bieter, using the less sensitive methods of his time, foud no protein in the urine of *Lophius* (*J. Pharm. and Exper. Therap. 43:406, 1931*). His finding that injected hemoglovin appeared in the urine of glomerular but not of aglomerular fish led to the conclusion that most urinar, protein was of glomerular origin.

A more valid examination of the renal handling c plasma proteins in aglomerular fish has been mad possible by the recent develoment of micro techniques polyacrylamide gel electrophoresis. The appearance c any plasma protein in the urine of an aglomerular fish is c interest with respect to its mode of access, secretory o otherwise. Moreover, since filtration and tubular reab sorption of low molecular weight proteins (LMW) are important processes in their catabolism in a filtering kidney we may question if accumulation in plasma o alternate pathways of disposal for LMW proteins hav evolved with the loss of glomerular function.

In this report data are presentd for the ren clearance of plasma proteins by *Lophius* and for tra clearance of an inert macromolecule (PVP) injecte intravenously.

Clearance experiments were performed in male fish weithing from 2000 to 3000 g. Fish were placed ventra side up with head and gills submerged. Both ureters we catheterized with PE tubing through a ventral abdomina incision. Blood samples were taken from the caudal vein Total protein concentration in plasma and urine wa measured by the Lowry method. Micro-polyacrylamide electrophoresis with a continuous gradient from 2 to 40% was performed on diluted plasma and unconcentrate urine samples. Some urine samples were concentrated b ultrafiltration in dialysis tubing (Union Carbide) an Amicon filters(UM 2). Concentrated urine or unconcen trated plasma was separated by gel filtration on Sephade G 100, G 75 and G 50 columns. Reconcentrated elute fractions were again separated by gradient gel electro phoresis. One fish was injected with PVP (MW 40,000 and renal clearance and plasma disappearance curve o macromolecules were determined.

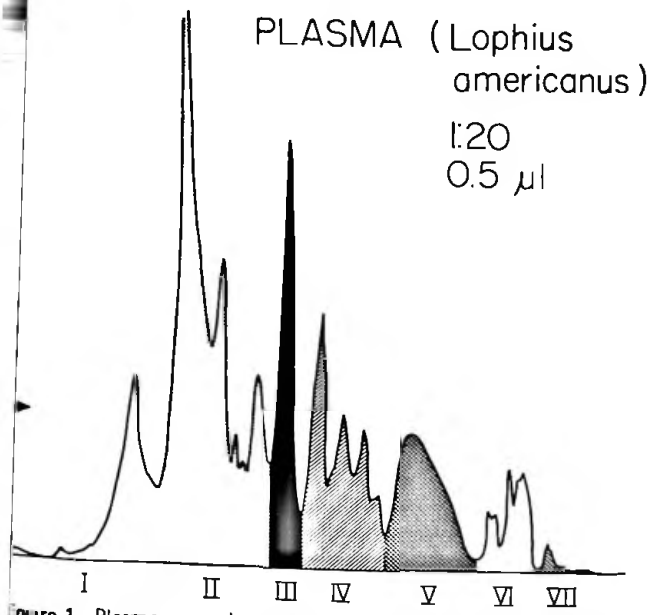

Figure 1 Plasma protein pattern in gradient-gel-electrophoresis (0.5 µl of 20x diluted fish plasma). The direction of migration is from left to right. Roman numerals indicate different fractions.

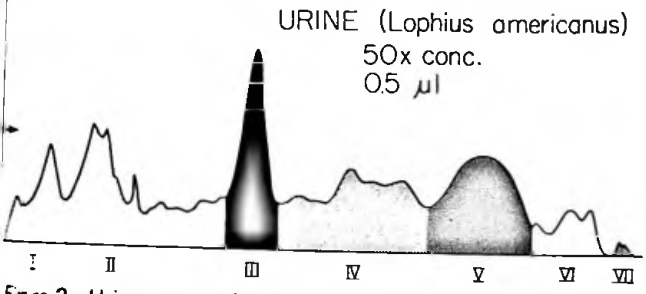

Figure 2 Urinary protein pattern in gradient-gel-electrophoresis (0.5 µl of 50x concentrated fish urine). Fraction numbers (Roman numerals) correspond to fraction numbers in Figure 1.

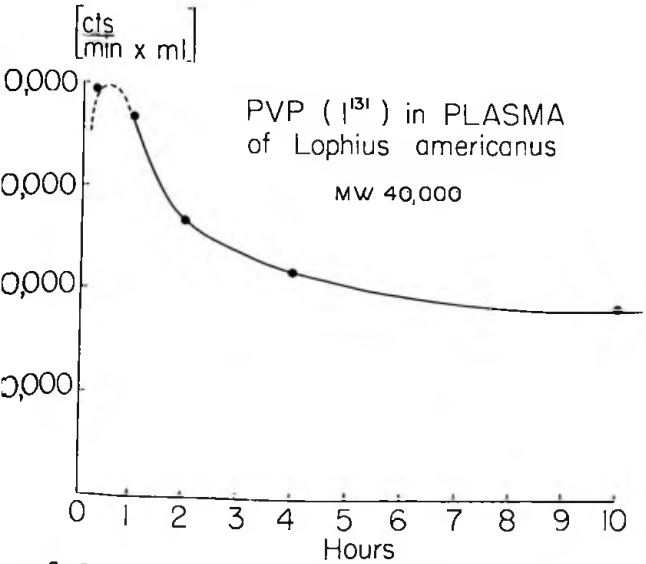

Figure 3 Disappearance curve of PVP from plasma.

Plasma total protein concentration was 2032±92 (SD, n=5) mg/100 ,l and urinary protein was 0.72±0.07 (n=19 mg/100ml.

Fig. 1 shows the pattern of plasma electrophoresis of 0.5 l of 20x diluted plasma. Proteins, 32 in number, were divided into 7 major fractions, according to their separation on Sephadex G100 gel. The MW range is =100 000(#1), 70 000(#2), 55 000(#3), 45 000(#4), 33 000(#5), 18-25 000(#6) and 11-15 000(#7). There is no redominant plasma protein band corresponding to albumin in mammalian plasma. The fraction of proteins smaller than 55,000 MW comprises 27.8±3.4%(n=5) in plasma. Fraction #3 (55,000) is 13.4±1.2% and higher molecular weight proteins are 59±2.8%.

Fig. 2 shows the pattern of urinary proteins in 0.5 l of 50x concentrated fish urine. The curve reveals the whole spectrum of plasma proteins, but at a very low concentration (total protein=0.72±0.07mg/100ml).

Table 1 shows the renal clearances of these proteins as calculated for one kidney and the renal clearances for PVP in the same kidney and time period. The slope for the disappearance of PVP from plasma in the same animal is plotted in Fig. 3.

Quantification of the different protein groups was performed b integrating the corresponding electrophoretic bands. Results are shown in Table 2. Fraction #3(MW 55 000) was used as reference for establishing a ration of molecules smaller than 55,000 (LMW proteins) and larger than 55,000.

Molecules smaller than 55,000 comprise in plasma 27.8±3.4%(n=5) and in urine 59±4.2%(n=3), whereas

Table I

$C_{PROTEIN}$ & C_{PVP} min^{ml} x 100g BW x 1 Kidney

	$C_{PROTEIN}$ Kidney		C_{PVP} Kidney	
t- Period	Left	Right	Left	Right
U2	$0.8\ 10^{-6}$	$0.5\ 10^{-6}$	$1.8\ 10^{-6}$	$2.7\ 10^{-6}$
U3	$0.6\ 10^{-6}$	$0.35\ 10^{-6}$	$2.5\ 10^{-6}$	$2.6\ 10^{-6}$
U4	$0.18\ 10^{-6}$	$0.62\ 10^{-6}$	$2.7\ 10^{-6}$	$3.0\ 10^{-6}$
U5	$0.45\ 10^{-6}$	$0.24\ 10^{-6}$	$2.1\ 10^{-6}$	$2.2\ 10^{-6}$
U6			$2.7\ 10^{-6}$	$2.3\ 10^{-6}$

Renal clearances of total protein ($C_{protein}$) and PVP (C_{PVP}) (i.v.; M.W. 40,000) for 2 kidneys of the same animal. (L3).

Table II

Fractions of Plasma Proteins in Gradient Gel Electrophoresis

	<55,000	=55,000	>55,000
Urine	59.0±4.2% (n=3)	19.0±1.5% (n=3)	22.0±5.0% (n=3)
Plasma	27.8±3.4 (n=5)	13.2±2.8 (n=5)	59.0±2.8 (n=5)

Percent values of proteins with higher and lower MW than 55,000. The fraction with MW 55,000 is identical to fraction III in Figure 1 and Figure 2.

the fraction of high molecular weight proteins (#1&#2) is $22 \pm 5\%$(n3) in urine and $59 \pm 2.8\%$(n5) in plasma.

Although the disappearance curve of PVP from *Lophius'* plasma (Fig. 3) follows a time course similar to that in mammalian species the renal clearance of this molecule is incomparably less. In the rat, for example, clearance of PVP (MW 40,000) is 10,000 x the value calculated here for *Lophius.* The extrarenal disposal of this macromolecule is therefore of considerable interest.

When relative clearances of PVP and protein are compared within species another striking difference is found. In *Lophius* (Table 1) the clearance of PVP is 3 or 4 x tht of total plasma protein, in the rat it is about 1000 x. This dixcrepancy may be explained by the relatively small molecular weight of fish plasma proteins and the large fraction of LMW proteins in the plasma of *Lophius.*

These preliminary data allow no more than inference regarding the mode of access of plasma roteins to the urine in this aglomerular species. The fact that all plasma proteins find urinary representatation speaks against secretion since it seems unlikely that secretory mechanisms for every protein would be evolved. Also LMW proteins predominate in urine whereas known secretory proteins (IgA,uromucoid) are of high molecular weight. It is possible that the very small rate of urinary protein excretion (12 g/hr/kidney) represents leakage of plasma proteins through intercellular spaces. The bulk flow of urine across these epithelial structures may assist the movement of proteins. The predominance of LMW proteins in the urine of *Lophius* is consistent with this process of proteinuria.

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Metabolism of the Standing Dead Plant Community in Several Maine Salt Marshes

John L. Gallagher and William J. Pfeiffer. The University of Georgia Marine Institute, Sapelo Island, Georgia

The salt marsh, like many natural grasslands, is a detritus based ecosystem. Relatively few grazers are present and most of the photosynthate enters the food web via microbial degradation. Microbes are active in southeastern United States marshes throughout the year, but in Maine a strong seasonality in plant decay would be expected.

Aerial and aquatic respiration of the dead plant communities and dissolved organic carbon (DOC) release were measured in stands of *Spartina alterniflora* Loisel., *Spartina patens* (Ait.) Muhl. and *Juncus gerardi* Loisel. Samples were collected from marshes at Northeast Creek and Hog Bay in Hancock County. Aquatic respiration of the community associated with the standing dead plants was measured as oxygen consumption by the Winkler method and aerial respiration was quantified as Co_2 release with an infrared gas analyzer. DOC discharge was measured by the method described by Gallagher *et al* (*Estuar. Coast Mar. Sci., in press*).

Aquatic respiration rates of the three dead plant communities were similar in April when the temperature was 1°C. They were 14.9($\pm$ 0.6), 12.6($\pm$ 1.0), 15.4(0.5) g C hr^{-1} gram dry $weight^{-1}$ ($\pm$ SE) for the *alterniflora*, *S. patens* and *J. gerardi* respectively. O the wide temperature range used to compare the Ma (ambient 1°C) and Georgia (ambient 25°C) communit the Q_{10} was 2.75. Aerial respiration rates in April w slightly lower than those measured under aqua conditions. In May the aerial Q_{10} for the *S. alterniflo* community averaged 3.89 over the temperature range 2-18°C, while that for *S. patens* was 2.77. These val are higher than similar values for Georgia and i indicate the saprophages in Maine communities adapted to take advantage of short periods of wa weather. Carbon dispersion from the dead pla communities by DOC leaching was more than 4 tim that of respiration rate. These compounds released fr the dead plant communities are readily absorbed planktonic heterotrophs. Chemical analyses of the d plant communities for sugars, starch and crude pro are not complete, but similar studies in Georgia ha shown a strong correlation between respiration rate a protein content of the dead plants.

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Free Amino Acids In Tissues Of The Skate, *Raja erinace* Regulation Of Concentrations And Transport Duri Adaptation To A Dilute Sea Water Environment.

Leon Goldstein, Thomas A. Boyd, Anne E. McElro Chung-Ja Cha and Roy P. Forster Brown University, a Dartmouth College

Study of intracellular osmoregulatory processes difficult in higher vertebrates because these anima not survive large changes in osmolarity of body flu However, certain fish (euryhaline elasmobranchs) a suited ideally for this type of study, for they tole relatively large alterations in body fluid osmol Previous studies (*Forster and Goldstein, Am. J. Psys in press*) have shown that amino acids play a major ro intracellular osmoregulation in the skate *Raja erinacea*; total free amino acids in skeletal muscle and RBC skates kept in seawater (SW) comprise more than percent of the osmotically active solutes, and th concentration is reduced significantly when the fish adapted to half-strength sea-water (½ SW). In the pres study, we measured the levels of individual amino acid RBC, wing muscle and heart of skates maintained in and ½ SW.

Blood was drawn from a caudal vessel. The sk was pithed and a piece of wing muscle and heart qui removed and immediately freeze-clamped with alumin blocks precooled in liquid nitrogen. Blood was separa into packed RBC and plasma and the latter deprotein with 10 percent trichloroacetic acid (TCA). The TCA removed with ether. Frozen, packed RBC, wing mu and heart were powdered separately in mor surrounded with dry ice and extracted with 9 vol percent sulfosalicylic acid. The tissue and plasma ext were dried under vacuum, over conc. H_2SO_4, at 40 The dried residues were dissolved in appropriate volu